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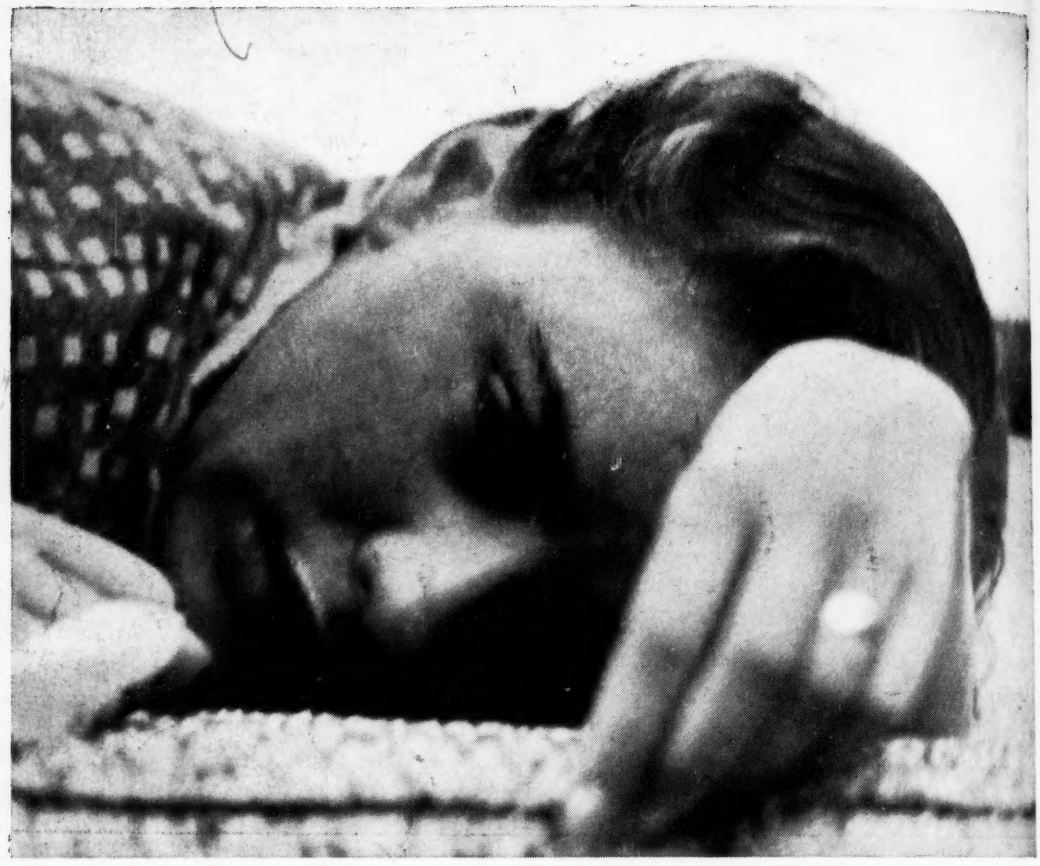

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The Journal of Allergy

VOL. 27 MAY, 1956 NO. 3

Original Articles

BACTERIAL HYPERSENSITIVITY

SIDNEY RAFFEL, M.D., STANFORD, CALIF.

DISCUSSIONS of the hypersensitive states usually follow a simplified category; these states are referred to as immediate or delayed in type. The former includes those allergic situations in which reactivity to antigen is likely to be rapid, in which a rather limited number of tissue types become involved in the reaction, and in which humoral antibody is demonstrably related to the reactive state. The delayed states encompass those which we also know by the terms "infectious hypersensitivity" and "contact allergy." In these, reactivity following exposure to antigen is relatively slow in appearing; there may be a wide range of cellular types involved in the reaction; and no conclusive evidence of relationship of humoral antibodies to these hypersensitivities has yet been put forward. It seems doubtful that in the long run these characterizations will remain intact. There is some question, for example, about the second point—whether in delayed hypersensitivity tissue reactivity is really as widespread as has generally been thought—and there is current question also as to whether or not an unusual kind of humoral antibody may be concerned in delayed hypersensitivity. Whatever time may bring forth in the way of clarification of these and other questions, the fact remains that the contact and infectious allergies do show certain striking differences from the immediate hypersensitivities, and consequently will probably always require special consideration.

Most of you here deal chiefly with the various manifestations of immediate hypersensitivity and, in the area of delayed hypersensitivity, with contact allergy in your practices. Unless I am much mistaken, few of you are ever directly concerned with the bacterial allergies, and this is so in part because these hypersensitivities are in a sense hidden among the multiple events transpiring during the courses of infectious diseases. In most such diseases it seems doubtful that hypersensitive reactivity plays an important role in the

From the Department of Medical Microbiology, Stanford University School of Medicine.

Read before the American Academy of Allergy, St. Louis, Missouri, Feb. 7, 1956.
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pathogenesis of the total infectious process; in the restricted instances in which it does, as in tuberculosis or lymphogranuloma venereum where tissue destruction is chiefly the result of such allergic reactivity, the medical responsibility falls outside your scope.

This means that the subject of bacterial hypersensitivity really represents for many clinicians an exercise in the intellectual effort to understand these processes, whatever their immediate implications with respect to therapy or control. I assume that it is chiefly for this reason that you have asked me to discuss this subject and, acting on this assumption, I shall try to present three of the principal facets of these hypersensitivities: first, the mechanism through which the bacterial hypersensitivities develop; second, the cellular origin of these immunologic responses; and, third, the tissues and cells concerned in the reactive state.

1. *The Induction of Bacterial Sensitivity.*—It has been known for many years that the delayed allergic states which accompany infectious processes cannot be reproduced by the isolated antigenic substances of the causative organisms. Conversely, it has also been known that animals infected, for example, with the tubercle bacillus and highly sensitive to constituents of this organism in the delayed fashion might frequently be anaphylactically totally unreactive.[1, 2] These observations indicate that the two states—the immediate anaphylactic and the delayed infectious—are not disposed to appear under the same circumstances of antigenic stimulation, despite the fact that they can both be responses to the same antigen. This may be restated in simplified experimental terms: if the proteins from the tubercle bacillus are injected into appropriate experimental subjects, these soon become anaphylactically sensitive but never sensitive in the delayed sense; conversely, if tubercle bacilli are introduced into experimental animals, they develop bacterial hypersensitivity to the same protein, and may or may not become anaphylactically sensitive also.

A few years ago we began to make a study of the various isolatable constituents of the tubercle bacillus in relationship to immunologic responses, and soon found that when bacilli had been deprived of lipids extractable by chloroform they simultaneously lost the ability to induce bacterial hypersensitivity, although the protein antigenicity of the treated bacillary bodies remained virtually intact. Upon re-adding extracted constituents to these defatted bodies, and administering the various combinations to animals, it was then found that a fatty substance characterized by Anderson[3] as a "wax" was essential for the sensitizing process, and further that the activity of this substance was of nonantigenic character; that is, the presence of this substance in the tissues simultaneously with antigenic proteins of the organism in some way causes the responding animal to develop the bacterial type of hypersensitivity. Subsequent and still continuing studies have brought us closer to appreciation of the nature of the active principle of the lipid; this consists of an 88 carbon fatty acid called mycolic acid esterified with a polysaccharide of molecular weight probably in the neighborhood of 8,000 to 10,000.[4-9] More

recent studies in conjunction with Drs. Lederer and Asselineau of Paris indicate further that this activity in the sensitization process is possessed by other esters of mycolic acid; for example, galactose or a trisaccharide mycolate, or even the simple methyl mycolate, will also act in this fashion.[10, 11] Furthermore, such lipoidal factors serve to direct sensitization to antigens unrelated to the tubercle bacillus, as, for example, egg albumin[12] and picryl chloride.[13] The mechanism through which the lipid plays its role is not yet known; whether it increases cellular permeability to antigen, stimulates the proliferation of certain cell types which may be responsible for the origin of delayed hypersensitivity, or simply stimulates existing cells to increased activity are questions which themselves require extensive discussion and which I have not included for consideration here.[14]

This chemical explanation for the development of one of the major bacterial hypersensitivities is unfortunately not readily transposable to hypersensitive states in the case of other infectious agents. During the past several years we have attempted to demonstrate a similar chemical-biologic relationship of lipids and antigens in the development of sensitivity to a hemolytic streptococcus, to the fungus *Aspergillus niger,* and to a virus, vaccinia. In all three instances there have been suggestive results, but in no case has there been the clear-cut delineation of this relationship so readily seen in the case of tuberculosis. This may be accounted for by our ignorance of the specific chemical nature of the substances for which we seek and improper manipulations in trying to remove them from cells, and it may depend in part upon the lesser degree of sensitization to these infectious agents than occurs in tuberculosis, with increased difficulty in assessing observations. In any case, our observations, though not definitive, have been sufficiently suggestive to keep us on this track.

2. *Origin of Delayed Hypersensitivity.*—The problem of cellular origin of bacterial hypersensitivity may be stated in this way: Are the cells which are responsible for the manufacture of humoral antibody also responsible for the development of bacterial hypersensitivity? If so, then the bacterial hypersensitive response may be considered a somewhat aberrant variant of the humoral antibody response, perhaps in the sense that atopic reagins possess distinctive peculiarities in relation to the more conventional serologically active antibodies. If not, then bacterial hypersensitivity may constitute a response which is fundamentally different from the humoral antibody response.

All of you know that there is no clear answer to this question now, but there are a number of interesting trails of evidence which may be converging upon the goal.

First, since we are considering the origin of delayed hypersensitivity on a basis of comparing this with the origin of humoral antibodies, we should briefly survey what is known about the source of antibodies. In past years it was generally thought that macrophages of the reticuloendothelial system synthesize antibodies, and a good deal of cogent evidence was advanced to support this viewpoint, including the fact that these cells take up foreign

material, soluble as well as particulate, that "blockade" of the phagocytic system results in suppression of antibody formation, and that macrophages observed while living appear to shed cytoplasm (possibly antibody globulin) and in tissue cultures appear to manufacture globulin.[15] This viewpoint has gone by the board for, although the ability of these cells to ingest foreign substances has never been in question, newer methods of searching for antibodies in tissues have failed to reveal them in macrophages.[16-19] About ten years ago the lymphocytes came into prominence in this respect, chiefly on the basis of evidence provided by the effects of adrenal cortical extracts upon antibody formation,[20, 21] and at about the same time evidence was adduced from cytologic studies of patients with myelomatosis and hypergammaglobulinemia,[22, 23] as well as from direct studies of antibody production that plasma cells may manufacture globulin and antibody.[24-28] The question as to the relative place and importance of these last two cell types in antibody manufacture has not yet been fully resolved. Lymphocytes appear to have some vital role in the total process of antibody synthesis, as indicated especially by the fact that cellular suspensions consisting almost entirely of lympho-

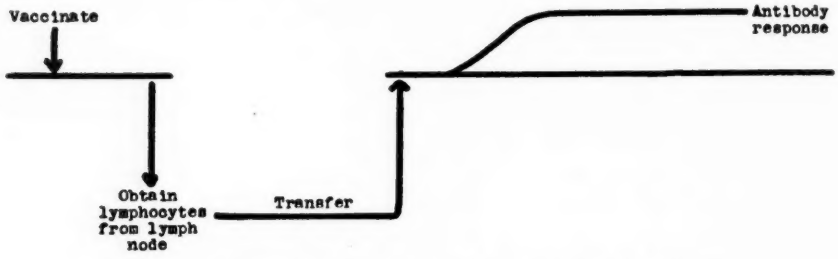

Fig. 1.—Evidence for lymphocyte origin of humoral antibody.

cytes teased from nodes of vaccinated animals can transfer to an irradiated recipient the ability to develop antibodies after an appropriate period (Fig. 1).[29-38] On the other hand, it is difficult to regard the lymphocyte as the ultimate cell concerned in antibody formation for several reasons: (1) because direct examinations of tissues of vaccinated animals by tracer techniques show the antibody to be in plasma cells,[16-19] (2) because patients with agammaglobulinemia who are unable to form antibodies are entirely lacking in plasma cells, even in lymph nodes regional to sites of vaccine injection[39] (Fig. 2), and (3) because of the extensive experiments with x-irradiation carried out during the past few years. These have revealed that, although total body irradiation applied in advance of an antigenic stimulus can be completely suppressive to the antibody response, there is a period of grace for some hours after irradiation during which administration of antigen can still be effective in eliciting almost the normal response (Fig. 3).[40-48] This is impressive in view of the general agreement among pathologists that the lymphocytes are among the earliest cells destroyed by irradiation, within hours after its application. It would appear, from this, that lymphocytes and antigens may need to be together in the body for a short period only, and that subsequently antibodies

may continue to be produced by some radio-resistant cell (possibly the plasma cell) at a time when almost all lymphocytes have been destroyed. Robertson[49] has observed in rabbit ear chamber preparations that cells resembling lymphocytes may take up soluble antigen in the first few hours after its administration, but lymphoid cells with ingested antigen were not seen after the first day. Other observations also indicate that protein antigens reside in lymphocytes only transiently and to a limited extent.[49a, and b]

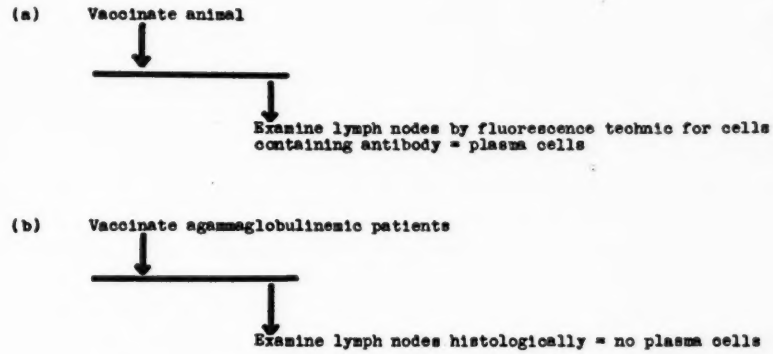
(a)
Vaccinate animal
Examine lymph nodes by fluorescence technic for cells containing antibody = plasma cells
(b)
Vaccinate agammaglobulinemic patients
Examine lymph nodes histologically = no plasma cells

Fig. 2.—Evidence for plasma cell origin or humoral antibody.

The essence of researches so far carried out indicates, then, that plasma cells must be involved in antibody production, and that lymphocytes also must be concerned in the process in some fashion, possibly at an early stage in the reaction between antigen and the tissues of the responding animal. On this tentative basis, we can proceed to examine the question of origin of bacterial hypersensitivity.

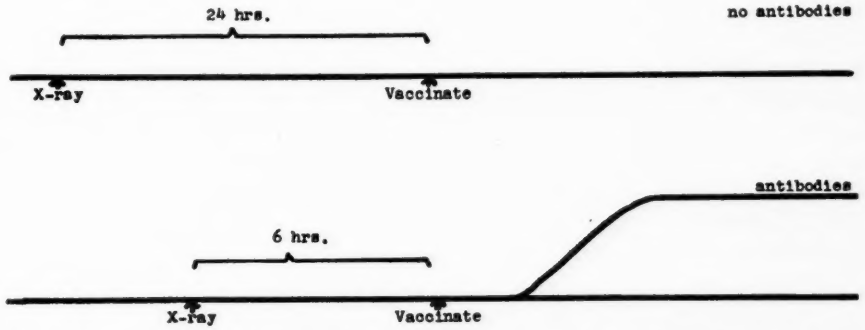

Fig. 3.—Evidence for possible preliminary role of lymphocyte in origin of humoral antibody.

A humoral antibody in bacterial hypersensitivity: For a good many years now, it has been the general opinion that the existence of bacterial hypersensitivity is not related to humoral antibody. There has been equally general agreement that features of this hypersensitivity—the period required for its induction, its specificity, and the occurrence of anamnesis—are sufficiently akin to features of humoral antibody development that some kind of cellular

antibody must be involved in this situation. Recently, Cole and Favour[50] reported that guinea pigs sensitized by killed tubercle bacilli possess an antibody which occurs in the alpha globulin of the plasma, and which can transfer to normal animals reactivity to tuberculin. They account for the failure of previous transfer studies with whole serum by the finding that the presence of gamma globulin, which contains antibodies of the "conventional" type to tuberculopolysaccharide, in some way inhibits the ability of the alpha fraction to transmit hypersensitivity. There is some precedent for the inhibition or masking of antibody activity by plasma components.[51, 52] If Cole and Favour's observations prove to be confirmable, they could in one sense settle the issue of origin of bacterial hypersensitivity by relegating this to the same cells which manufacture other humoral antibodies; on the other hand, the fact that the sensitizing antibody resides in an unusual chemical portion of the plasma could well mean that its origin is not the same as that of antibodies of gamma globulin composition. For the present, at least, the observations of Cole and Favour cannot supply direct evidence upon the point in question. Other avenues, followed above in discussing the origin of humoral antibody, have been investigated also in relation to bacterial hypersensitivity.

Passive transfer: Turning now to studies of passive transfer of hypersensitivity by cells, we can compare what has been found here with observations made in the case of humoral antibodies. It has been known for a number of years, since early work of Bail[53] and later, more definitive studies by Chase[54] and others,[55-57] that cells obtained from subjects with bacterial hypersensitivity can transmit this reactive state to normal recipients. In more recent work, cells teased from lymph nodes and consisting mainly of lymphocytes have been found to transmit the sensitivity, just as is true of humoral antibody.[58] On the surface, this coincidence seems to point directly to the conclusion that humoral antibodies and bacterial hypersensitivity must have a common cellular origin. However, certain observations do not permit so direct a conclusion.

Keeping in mind the tenuous state of knowledge about the lymphocyte's role in serum antibody production to begin with, several considerations give pause to acceptance of the idea that bacterial hypersensitivity arises from the same cells, or even via the same pathways, as do plasma antibodies.

In the first place, there may be fundamental differences between the transfer by lymphocytes of a potential ability to produce antibodies and of bacterial hypersensitivity. In the former instance, various studies referred to previously indicate that the transfer is not a direct passive one of antibody already existent in the cells but, rather, of a potential ability. That is, once cells have been planted in the recipient, there follows a *developing curve* of concentration of antibody in the plasma, a process akin to active production.[29-38, 59] In the case of transfer of bacterial hypersensitivity, on the other hand, there is no real evidence of a similar progressive development in the recipient. Hypersensitivity appears rapidly and in toto, remains for its evanescent period, and disappears.[60] (Lawrence[61] has found in human beings

that hypersensitivity to tuberculin and streptococcal products may be transferred by leukocytes, and that the reactivity in recipients arises late and may persist for months. This is in contrast to observations made in experimental animals, and has yet to be interpreted in this respect.) This contrast is graphically suggested by Fig. 4. This could mean that in the case of bacterial hypersensitivity the transferred cells simply bring over to the recipient their own already existing sensitization, and themselves react with the antigen employed to test the recipient. Information concerning the life span and the distribution in the recipient of transferred cells is consonant with this possibility.[60] If this is the case, the lymphocytes may represent simply one kind of sensitized cells of the allergic donor, rather than necessarily an origin of the hypersensitive state.

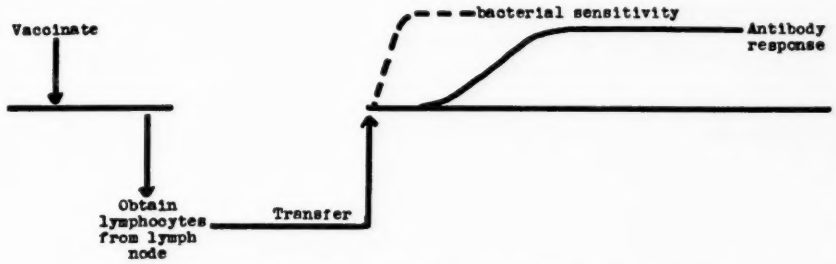

Fig. 4.—Evidence for lymphocyte origin of bacterial hypersensitivity.

If transferred bacterial hypersensitivity represents merely reactivity of the passaged cells in a new individual, one would expect that other sensitive tissues and cells might function like lymphocytes in this respect. There have been few trials of this point—Chase and his associates[58] state that monocytes collected from the peritoneal cavity probably lack the ability to transfer delayed hypersensitivity, and Kirchheimer and associates[62] have failed also with granulocytes in guinea pigs. However, Lawrence[61, 63] has successfully employed blood leukocytes for the purpose, and Schlange[64] has reported the transmission of tuberculin sensitivity via transfusions, in both cases in human beings. Obviously, lymphocytes were included among the cells transferred in these latter instances, but as minority constituents.

That some essential difference may exist between the cellular transfer of humoral antibody synthesis and of bacterial hypersensitivity is suggested also by the experiments of Good and Varco[65] in human subjects. The transfer of white cells from typhoid-paratyphoid-vaccinated normal donors to patients with agammaglobulinemia failed to induce in the recipients any circulating antibodies to these bacteria, while, on the other hand, the recipients did develop the bacterial hypersensitivity to streptococcal antigens which the donors had also possessed. This again implies that, in the case of bacterial hypersensitivity, cells may transfer an already existing state to a recipient who, because of lack of elements essential for completing the synthesis of humoral antibodies, is incapable of carrying forward this process.

Related to Good and Varco's experiment in the sense of revealing that the phenomena transmitted by cells to a recipient may be dissociable are the observations of Chase[58, 59] in guinea pigs. If animals are sensitized with picryl chloride contained in an oily mixture in which killed tubercle bacilli are incorporated, they develop antibodies, anaphylactic sensitivity, and contact sensitivity to the picryl chloride, as shown in Fig. 5. (Contact sensitivity is generally considered an analogue of bacterial hypersensitivity.) The lymphocytes of these animals transfer to normal recipients all three of these properties. If, on the other hand, animals are given picryl chloride conjugated with protein, without the presence of tubercle bacilli in the sensitizing mixture, only the antibody and anaphylactic responses develop, as is well known. Lymphocytes from these animals transfer only these two kinds of reactivity to normal donors. In both experiments the donor animals are engaged in

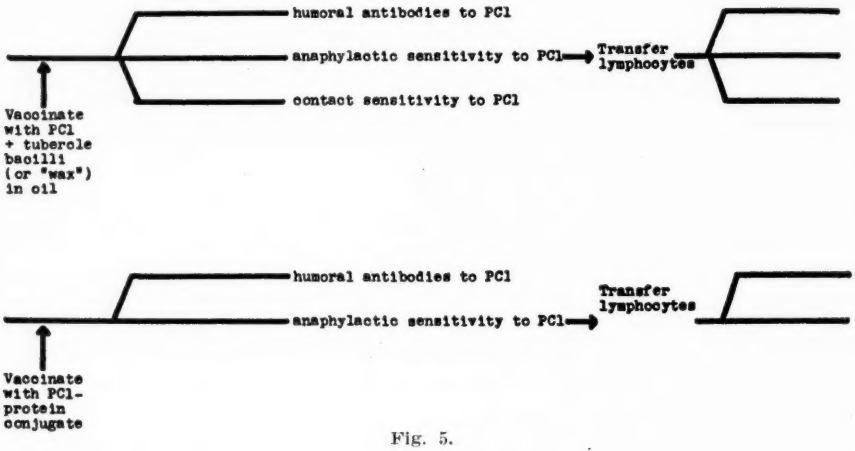
humoral antibodies to PCl
anaphylactic sensitivity to PCl
contact sensitivity to PCl
Transfer lymphocytes
Vaccinate with PCl + tubercle bacilli (or "wax") in oil
humoral antibodies to PCl
anaphylactic sensitivity to PCl
Transfer lymphocytes
Vaccinate with PCl-protein conjugate

Fig. 5.

responding immunologically to a single chemical antigenic determinant; if in both cases the same cellular elements take part in the process, it is difficult to understand why, on transfer to recipients, these cells persist in transmitting only those responses of which the donors are capable. There must be some qualitative difference in the roles of these cells in the two instances.

Agammaglobulinemia: Studies of agammaglobulinemic patients have been mentioned in respect to passive transfer of bacterial hypersensitivity, but there is another aspect of origin to which data on such subjects may contribute. Since these patients are unable to produce antibodies, at least of the "conventional" kind (apparently no studies have been made of their ability to manufacture atopic reagins, which occur chiefly in the beta globulins[66-70]), it would be very pertinent to this discussion to inquire whether they are able to develop bacterial hypersensitivity. If they can do this in the absence of the ability to acquire plasma antibodies, the evidence would be strong for a dissociation of origin of these two entities.

The subjects studied by Good and Varco[39] were subjected to skin tests for hypersensitivity to the streptococcus, and the reactivity which is expected to occur in 80 per cent of normal subjects was absent in the agammaglobulinemic patients. In the case of tuberculin testing, however, the results were not so conclusively negative. Of seven patients tested, one developed erythema persisting for seventy-two hours after even a 1:10,000 dilution of Old Tuberculin, but this reaction was not accompanied by induration. These investigators decided, in view of the absence of reactivity to the streptococcus and of the atypical character of the tuberculin reaction in the responding patient, that agammaglobulinemic subjects fail to develop bacterial hypersensitivity. Again it would seem wise, for the present, to leave the decision open pending more information for, although there is undoubtedly a decreased incidence of hypersensitive states in these patients, these may occur nevertheless. I have learned recently of two other instances of positive reactivity to tuberculin in agammaglobulinemic subjects—one in a 35-year-old man observed by Drs. H. H. Zinneman and W. H. Hall of the Veterans Hospital in Minneapolis, and a second in a child studied by Drs. Edith Lincoln and Herbert Porter at Bellevue Hospital. Zinneman and Hall's patient lacked gamma globulin and all humoral antibodies, as indicated by the absence of isohemagglutinins. The child studied by Lincoln and Porter was not completely lacking in gamma globulins but its concentration was less than 1 per cent of the normal value. In this case, vaccination with BCG was followed after ten weeks by a positive reaction to Old Tuberculin in 1:10,000 dilution.

Suppressors of immunologic responses: A final line of evidence which bears upon the question of the origin of delayed hypersensitivity comes from studies of the influence of various suppressive agents, including x-irradiation, nitrogen mustard, and cortisone, upon immunologic responses. It seems clear, from the extensive work referred to earlier, that the exposure of animals to total body irradiation at appropriate periods before exposure to antigens may completely suppress the humoral antibody response (Fig. 3). Similarly, Lennox and co-workers[71] have found that irradiation of rabbits just before inoculation of tubercle bacilli postpones the occurrence of tuberculin sensitivity. From such information, one might conclude that irradiation affects a process common to the elaboration of bacterial hypersensitivity as well as humoral antibody, but once again there is cause for question. In addition to the effect described, these investigators noted an influence of irradiation upon hypersensitivity which is not seen in the case of serum antibodies, namely, that exposure of animals to x-rays *after* tuberculin sensitivity had developed resulted in the suppression of visible reactivity to the test antigen. (Surprisingly, histologic sections of tuberculin-tested areas revealed inflammatory responses which could not be differentiated in intensity from those appearing under ordinary conditions.) Recent work with guinea pigs[72] also demonstrates that tuberculin reactivity is diminished in irradiated animals receiving cells from actively sensitized donors. In both instances, loss of the dermal manifestations of bacterial

hypersensitivity occurs in circumstances which do not interfere with the appearance of serum antibodies. One may question, then, whether the suppressive influence of prior irradiation upon the development of bacterial hypersensitivity may be due to some effect other than immunologic interference of the kind exerted upon serum antibody synthesis. Whatever the nature of this effect, Lennox and associates[71] demonstrated that it is not direct radiation injury to the skin.

Like x-irradiation, nitrogen mustard has been shown by Orris and Eisen[73] to interfere with the development of tuberculin hypersensitivity, and the partially suppressive influence of cortisone in this respect is well known. Whether one or multiple aspects of immunologic responses are affected by such agents or whether effects upon nonimmunologic mechanisms (for example, the general anti-inflammatory activity of cortisone) accounts for such observations is not yet known.

In my opinion, these five facets of evidence bearing upon the origin of bacterial hypersensitivity do not necessarily disprove the contention that the cellular origin and development of bacterial hypersensitivity is equatable with that of humoral antibodies. In each instance, however, there are seen to be factors contradictory to such an equation, and these must be clarified before the general question can be settled in either direction.

3. *Tissue Reactivity in Bacterial Hypersensitivity.*—The final problem to be considered is that of extent of generalization of cellular reactivity in bacterial hypersensitivity. The status of this situation is, unfortunately, also sufficiently unsettled now as to permit only a recounting of existing evidence and an expression of personal opinion. The question as to which cells of the sensitized body are reactive to antigen is, in one respect, closely bound to that of the cellular origin of the hypersensitive state, for if it should eventuate that only one cell type is responsive to antigen, as one viewpoint proposes with respect to the lymphocyte, this would constitute strong evidence that this is also the cell in which hypersensitivity has its nascency. The latter consideration, however, will not be dealt with again here; this section will be concerned only with evidence regarding the hypersensitive reactivity of various tissues.

For some years, and especially since the writings of Rich,[1] it has been widely held that there is a general reactivity of tissues in the tuberculin type of hypersensitivity, with virtually all cells susceptible to injury upon exposure to antigen. This feature, in fact, has been considered one of the main points of distinction between the delayed and immediate types of reactivity, for in the latter more specialized "shock tissues" are involved. More recently evidence has appeared to question this viewpoint.

The work which led to the belief in generalized reactivity was carried out, for the most part, by exposing explanted cells of sensitive animals in

tissue culture to quantities of tuberculin or other appropriate antigen shown to be harmless to normal cells, and gauging the results in terms of cell migration and injury. A number of experimenters demonstrated, in addition, that cells from animals with immediate hypersensitivity to the same antigen were not injured under the same experimental conditions. For many of these tests, portions of hematopoietic tissue were used, commonly bone marrow or spleen. More recent affirmations of these results have been made with leukocytes and splenic tissue of guinea pigs,[74, 75] with liver cells,[76, 77] and with macrophages[78] and leukocytes[79] from tuberculous human beings. It bears mentioning, in this connection, that another type of test adds testimony to the concept that various cells of the hypersensitive body may be reactive to antigen. Antigen injected into the cornea, a tissue without blood supply and composed of specialized cell types, evokes distinct reactions in animals with bacterial hypersensitivity, but not in those having only serum antibodies directed against the same antigenic substance.[1, 6, 12, 15]

The opposing viewpoint—that hypersensitive reactivity is limited in its cellular distribution—supposes, of necessity, that the special cell type which does become sensitized lends its reactivity to other tissues. This would explain, for example, how dermal reactions can occur even though only lymphocytes are responsive to antigen. In some quarters lymphocytes and, in others, cells of mesenchymal origin are thought to partake in sensitization.[80, 81] The process by which such cells are supposed to provide reactivity to other tissues is either through liberation of contained antibody or, by virtue of their potential ubiquity, by themselves reacting with antigen in whatever area a test injection may be deposited.

The experimental evidence supporting this viewpoint is more varied in nature than that on the other side of the question. Although direct tissue culture tests have been employed here also, with negative findings in the case of bone marrow[82] and epithelial cells of liver and kidney,[83, 84] other modes of study have included determinations of the effect of antigen upon the respiratory rates of sensitized and normal dermal cells in vitro,[80, 81] assays of release of lysozyme from leukocytes as a measure of injury,[85] and visual as well as respirometric and glycolytic observations of cells from pleural exudates induced by injections of Old Tuberculin into sensitized and normal animals.[86] In all these cases little or no difference could be found as between cells from sensitive or normal donors.

Clearly, discussion of this problem at this time can do no more than bring to the surface the difficulties involved in arriving at conclusions. As is always the case when results of a negative nature are under consideration, the burden of proof remains with those who have failed to find a clear explanation in cytologic studies for the phenomenon of general tissue reactivity elicitable in the intact animal.

SUMMARY

In general résumé, the induction of one form of bacterial hypersensitivity, that to the tubercle bacillus, appears to depend upon the simultaneous activity of two bacillary constituents—the protein antigen and a fatty acid ester. In some as yet unknown way, these substances evoke in the normal subject a response to the antigen which we recognize as the bacterial type of hypersensitivity.

The avenue of immunologic response in this case probably will not turn out to be the same as that which leads to the appearance of humoral antibodies. It seems reasonable to believe that in the latter case lymphocytes may be involved in a preliminary stage of the response, with plasma cells later fabricating the finished product. In the case of bacterial hypersensitivity certain experiments, such as those involving passive transfer by cells, suppression of response by x-irradiation and other agents, and observations of agammaglobulinemic patients, suggest that the process may be the same, but in all these instances close inspection discloses sufficient contradictory information to hinder acceptance of this viewpoint.

Finally, the extent of involvement of various tissue and cell types in the reactive state is not clear. The fact that hypersensitive subjects respond locally as well as systemically to antigen in the absence of circulating antibodies gave rise quite naturally to the view that a widespread reactivity of cells is a characteristic of bacterial hypersensitivity. Pointed studies of this possibility in isolated tissue preparations have supported this viewpoint in the past, but recent contradictory experiments bring it into question. Considerable study will be required for the clarification of this and related questions.

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THE IMMUNOPHYSIOLOGY OF SERUM SICKNESS

Alterations in the Radiosodium Space and Serum Electrolyte Concentrations During the Course of Repeated Injections of Horse Serum Into Rabbits

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THE physiologic changes induced in experimental hypersensitivity, particularly those related to alterations in the distribution of body fluids and electrolytes, are poorly understood. Previous studies on experimental and clinical serum sickness, however, have suggested that immune reactions may be related to changes in plasma and extracellular fluid volumes—changes which were believed to be due to alterations in the permeability of capillary and cell membranes, respectively.[1-3] In these earlier publications, the physiologic effects produced by a single sensitizing dose of antigen and by the passive transfer of a single dose of antibody and antigen were reported.

The natural history of clinical allergic states, however, is one of repeated exposure to antigens. The physiologic changes induced thereby may differ quantitatively or qualitatively from those which follow a single sensitizing dose. This usual clinical sequence of events can be approximated experimentally by the repeated injection of antigen, and the physiologic changes so induced can be measured serially by techniques employing radioisotopes and flame photometry.

In the present study, experimental serum sickness in the rabbit has been used as a laboratory model of the classic anaphylactic type of hypersensitivity reaction. As the animals were subjected to multiple specific antigenic stimuli administered by various routes, serial determinations of the serum electrolyte concentrations and the radiosodium space were performed and an attempt was made to correlate the physiologic changes with the humoral antibody response.

MATERIAL

Six domestic rabbits of mixed breeds, weighing 2.5 to 3.5 kg. each, were placed in individual metabolism cages and were fed a stock diet, supplemented once weekly by fresh green vegetables. Water was given without restriction.

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The same pool of sterile horse serum, without preservative, was used throughout the study as the sensitizing antigen and as antigen for the quantitative precipitin test.

METHODS

General Plan.—The general plan of the experiment was to hyperimmunize the rabbits initially by the repeated intravenous injection of large doses of horse serum, and subsequently to challenge them with small doses of antigen administered parenterally. The changes in radiosodium space and in the serum sodium and potassium concentrations and antibody nitrogen titer were observed throughout this period, and an attempt was made to correlate these alterations with the immunologic findings. Toward the end of the period of observation, cortisone acetate was administered, in order to test its effects. At the termination of the experiment, tissue analyses were performed in order to determine the effect of the experimental procedures on the radiosodium and water contents of the tissues.

Laboratory Procedures.—The *radiosodium (Na^{24}) space* was determined as follows: A known volume of a physiologic saline solution, containing approximately 20 μc of Na^{24} per milliliter, was injected intravenously into a marginal vein of the ear. One hour later, when the equilibration point for radiosodium was reached, a single blood sample was drawn. The radioactivity was determined with a dipping tube and a scaling circuit; counts were made to 1 per cent accuracy. The radiosodium space was calculated from the following formula: volume of Na^{24} space in milliliter = (total counts/min. injected)/(counts/min./ml. serum). All values were corrected for radioactive decay. The standard solution which was used for injection was appropriately diluted with distilled water to approximate the counting rate of the serum. No correction was made for urinary excretion of the radiosodium during the period of observation, since the amount excreted was negligible.

Serum sodium and potassium determinations and *tissue analyses* were performed by methods previously described.[4] The *antibody nitrogen concentration* was determined by the method of Solotorovsky, Porter, and Silber.[5]

Experimental Procedure.—Except for one animal which died on the 103rd day, observations and measurements were made on the animals for 211 days. The animals were weighed frequently throughout the experiment.

After the base line determinations of the Na^{24} space, serum sodium and potassium concentrations, and antibody nitrogen titers were made, each animal was given intravenous injections of horse serum (1 ml. per kilogram of body weight) on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, and 24, for a total of eleven injections. No further injections of antigen were made until the fifty-second day. During this period the entire battery of measurements was repeated on days 16, 30, and 45.

On the fifty-second and fifty-sixth days, respectively, 1 and 2 ml. of antigen was administered to each animal subcutaneously, and the measurements were made twenty-four hours after each challenge dose. Three intradermal

challenge inoculations, consisting of 2 ml. of antigen per animal, were given on the sixty-fifth, seventy-ninth, and eighty-fifth days. All measurements were repeated twenty-four hours after each challenge, and also on days 72, 93, 100, 107, 114, and 120. On the 135th day, 2 ml. of antigen was administered subcutaneously to each animal, and the measurements were repeated on days 136, 141, 148, 162, and 169.

On day 174, each of five animals received 2 ml. of horse serum subcutaneously and 25 mg. of cortisone acetate intramuscularly. All measurements were repeated at twenty-four hours, and no cortisone was given on this day. Daily from the 176th through the 181st day, each animal was given 6 mg. of cortisone acetate by intramuscular injection, for a total of six doses. Chemical and isotopic measurements were made on days 190, 197, and 204.

The experiment was terminated on the 211th day, when the animals were killed by air embolism one hour after the injection of a large dose of radiosodium. Tissues were analyzed for radiosodium and water content by methods previously described,[4] and the mean values were compared statistically with those obtained in normal, nonsensitized rabbits.[6]

Statistical Methods.—The results of the blood studies were analyzed statistically by comparing the base line values with the experimental values in each animal, so that each animal served as its own control. The mean differences at each time interval were then obtained, and the significance of the mean differences was calculated from the following formula: $t = \frac{\bar{d}}{s_{\bar{d}}}$ $s_{\bar{d}} = \sqrt{\frac{S\,d^2 - \frac{(Sd)^2}{n}}{n\,(n-1)}}$

The data from the tissue studies were analyzed statistically by means of the following formula for the comparison of two groups with a different number of observations in each group:

$$t = \frac{\bar{x}}{s_{\bar{x}}} \qquad \bar{x} = \bar{x}_1 - \bar{x}_2$$

$$s_x = \sqrt{S^2\,(n_1 + n_2)/(n_1 n_2)}$$

$$S^2 = \frac{(\text{sum } X^2 - \frac{[\text{sum } X]^2}{n})_1 + (\text{sum } X^2 - \frac{[\text{sum } X]^2}{n})_2}{(n_1 + n_2 - 2)}$$

Degrees of freedom = n–2.

The calculated value of "t" was compared with the tabled value at (n–1) degrees of freedom, and the significance has been expressed in P values.[6]

RESULTS (TABLE I)

Initial Sensitization.—There was a slight progressive decrease in body weight during the period of sensitization. On the day that the multiple intravenous injections of antigen were completed (twenty-fourth day of the experiment), the mean body weight was 2,867 grams—6.7 per cent less than the mean base line value. With cessation of the injections, the mean body weight rose by the forty-fifth day to 3,035 grams, as compared with a base line value of 3,074 grams.

TABLE I. ALTERATIONS IN BODY WEIGHT, SERUM ELECTROLYTE CONCENTRATIONS, AND RADIOSODIUM SPACE IN RABBITS GIVEN REPEATED INJECTIONS OF HORSE SERUM

DAY OF OBSERVATION	WEIGHT (KG.)	RADIOSODIUM SPACE (ML.)	RADIOSODIUM SPACE (ML./KG.)	SERUM CONCENTRATIONS (MEQ./L.) SODIUM	SERUM CONCENTRATIONS (MEQ./L.) POTASSIUM	HUMORAL ANTIBODY CONCENTRATION (MG. N/ML.)	CHALLENGE DOSE OF HORSE SERUM*
Base line	3,074	845	275	144	5.3	0	
16	2,872‡	738‡	257	156‡	5.1	1.96	
30	2,877‡	803	280	148	4.6‡	3.24	
45†	3,035	836	276	150	5.3	1.07	
53	2,910‡	780	268	143	5.1	0.83	1 ml. subcutaneously on day 52
57	2,645‡	783	277	158	5.5	0.88	2 ml. subcutaneously on day 56
66	2,760‡	882	321‡	136‡	4.6	0.93	2 ml. intradermally on day 65
72	2,597‡	694‡	268	153	4.9	1.09	
80	3,006	857	286	147	4.7‡	0.26	2 ml. intradermally on day 79
86	2,658‡	822	309‡	143	4.8	0.34	2 ml. intradermally on day 85
93	2,557‡	795	311‡	152	5.1	0.93	
100	2,268‡	687‡	304‡	153	5.4	0.37	
107	2,545‡	940	370‡	137‡	5.4	0	
114	2,662‡	780	280	144	5.2	0	
120	2,452‡	652‡	266	134‡	4.8	0	
136	2,177‡	796	340‡	147	5.1	0	2 ml. subcutaneously on day 134
141	2,482‡	743	303	149	5.0	2.06	
148	2,374‡	726	309‡	154	5.2	0.91	
162	2,631	796	305‡	154	5.1	0.47	
169	2,684‡	696	260	144	5.4	0.39	
175	2,446‡	606	250	143	4.9	0.30	2 ml. subcutaneously with cortisone, 25 mg., intramuscularly on day 174; cortisone, 6 mg. daily, on days 176 through 181
190	2,351‡	677‡	291	142	4.8	0.63	
197	2,264‡	694‡	308	150	4.8	—	
204	2,331‡	714	310	151	5.6	—	

*1 ml./kg. of horse serum was administered intravenously to each animal on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, and 24, for a total of eleven injections per animal.

†Measurements on day 45 were used as base line values for comparison with all subsequent measurements.

‡Statistically significant difference (P = less than 0.01).

Comparison of the mean base line values with those obtained on the sixteenth day of the experiment (after seven intravenous doses of horse serum) revealed the following statistically significant changes: a decrease of 202 grams in weight, a decline of 107 ml. in the Na^{24} space, and an increase of 12 meq. per liter in the serum sodium concentration. The decrease in weight persisted on the thirtieth day of observation, at which time the serum potassium concentration was decreased. On the forty-fifth day of observation, all values were similar to those observed prior to sensitization.

The mean serum antibody titer rose progressively from 0 to 1.96 mg. of antibody nitrogen per milliliter of serum on the sixteenth day of observation

and to 3.24 mg. N/ml. on the thirtieth day. It subsequently declined to values of 1.07 and 0.83 mg. N/ml. on the forty-fifth and fifty-third days, respectively.

Intradermal and Subcutaneous Challenges.—The measurements on the forty-fifth day have been used as base line values for comparison with all subsequent measurements. During the period between the forty-fifth and the seventy-second days of observation, while three parenteral doses of antigen were administered, the mean serum antibody nitrogen titer remained between 0.83 and 1.09 mg. N/milliliter. Except for the reduction in mean body weight, no other significant changes were noted until the sixty-sixth day, when the value for Na^{24}/kg. rose significantly at the same time that the serum sodium concentration decreased by 14 meq. per liter.

After the seventy-second day, the mean serum antibody concentration decreased to the point that none was demonstrable by the 107th day. Associated with a significant reduction in weight during this period were significant elevations of the values for Na^{24}/kg. on days 86, 93, 100, and 107. On the 107th and 120th days, the serum sodium concentration was decreased.

TABLE II. TISSUE RADIOSODIUM SPACE AND WATER CONTENT IN FIVE RABBITS ON THE 211TH DAY OF OBSERVATION, AFTER REPEATED INJECTIONS OF HORSE SERUM

TISSUE	TISSUE RADIOSODIUM SPACE (PER CENT OF WET WEIGHT)	TISSUE WATER CONTENT (PER CENT OF WET WEIGHT)
Skeletal muscle	9.91	81.41*
Spleen	18.32	82.83
Liver	18.98	75.64*
Stomach	21.36	81.60
Adrenal	23.04	—
Diaphragm	24.02*	81.32*
Appendix	24.05	84.19*
Gastrocnemius muscle	28.73	81.05*
Heart, ventricle	29.30	81.53
Lung	36.04	82.30*
Tendon	36.76	67.45*
Heart, auricle	38.03	82.69
Kidney	47.02	82.24*
Cartilage	48.87	67.37
Skin	50.69	79.60*
Subcutaneous connective tissue	59.49*	79.52*

*Statistically significant difference when compared with the mean values in a group of uninjected normal control rabbits[4] (P = less than 0.01).

After a lapse of about fifty days, when the serum antibody concentration was 0, 2 ml. of antigen was given subcutaneously on the 134th day. Two, fourteen, and twenty-eight days later (days 136, 148, and 162, respectively), the values for Na^{24}/kg. were again significantly elevated. No such significant change in the value for Na^{24}/kg. was noted on the 141st day, at which time the serum antibody concentration had risen to a mean value of 2.06 mg. N/ml. The antibody titer subsequently decreased progressively to a value of 0.30 mg. N/ml. on the 175th day.

Subcutaneous Challenge With Added Cortisone.—On the 175th day, the subcutaneous administration of 2 ml. of antigen was followed by daily doses of cortisone intramuscularly. The increase in antibody titer from 0.30 to 0.63

by the fourteenth day after injection (190th day of observation) was less than the rise in antibody titer obtained between days 136 and 148 (from 0 to 0.91 mg. N/ml.), when the same dose of antigen was given without the associated administration of cortisone. No significant change in the mean values for Na^{24}/kg. was observed from the 176th to the 204th day of observation.

Tissue Analyses (Table II).—The diaphragm and the subcutaneous connective tissue of the sensitized animals showed significant increases in the radiosodium space, as compared with uninjected control rabbits.

Significant increases in the tissue water contents of the appendix, kidney, gastrocnemius muscle, lung, diaphragm, liver, skin, subcutaneous connective tissue, tendon, and skeletal muscles were noted in the test group of animals.

COMMENTS

The most consistent finding in the present study was a reduction in body weight during the period of sensitization. Although the body weight returned to base line values following the initial course of antigen, it again decreased significantly during the period of antigenic challenge.

The simplest explanation for the changes observed during the first twenty-four days of the experiment would be that a reduced intake of food and water had occurred and that the transient increase in the serum sodium concentration was due to dehydration.

When the sensitized animals subsequently were again exposed repeatedly to the specific antigen, weight loss again occurred. The fact that this was now associated with an increase in the values for Na^{24}/kg., however, suggests that the composition of the body fluid compartments was now being altered in a different manner. A reduction in the radiosodium space which resulted purely from a decrease in body weight would be reflected by an insignificant change in the ratio of Na^{24} space to body weight. Such a change might occur as the result of a relative, generalized increase of catabolic over anabolic processes. On the other hand, a significant reduction in the serum sodium concentration or an increase in the value for Na^{24}/kg. suggests a decrease in the osmolarity of the extracellular fluid space and an alteration in the distribution of fluids among the different body compartments.

The simplest explanation for a rise in the value for Na^{24}/kg. is that there had occurred an increase in the volume of fluid in which the radiosodium ion was diluted. This could be due to a true increase in the volume of the extracellular fluid compartment, such as might result from glomerulitis or glomerulonephritis which is a prominent part of serum sickness in the rabbit. On the other hand, it may be an apparent increase resulting from an abnormally rapid exchange of the radiosodium ion with the fluid located intracellularly. The latter explanation implies that an abnormal increase in the permeability of the cell membrane was associated with an in vivo antigen-antibody reaction. However, insufficient immunologic data are available to establish a definite relationship between the immunologic phenomena and the physiologic changes. The increase in the value for Na^{24}/kg. was eventually associated with a

significant reduction in the extracellular concentration of serum sodium (at the 108th day). The physiologic alteration at this time may be interpreted as the result of a decrease in both intra- and extracellular osmolarity, with associated intracellular overhydration.

Cortisone, in the manner and dosage used, appeared to suppress both the humoral antibody response and the physiologic alterations.

The tissue analyses at the termination of the experiment showed that the most consistent increase in the Na^{24} space occurred in the diaphragm and in the subcutaneous connective tissue. The water content of most of the tissues examined was increased.

SUMMARY

Rabbits were hyperimmunized by repeated intravenous injections of horse serum, and serial measurements were made of the serum sodium and potassium concentrations, radiosodium space, body weight, and antibody nitrogen concentrations over a period of 211 days. The physiologic effects of intradermal or subcutaneous injections of small doses of antigen were observed repeatedly. The effect of the administration of cortisone was also observed. Finally, tissues were analyzed for radiosodium and water content.

Repeated challenges of the hyperimmunized rabbits with horse serum resulted in a decrease in the serum sodium concentration and an increase in the value for Na^{24} space as related to body weight. Cortisone appeared to suppress both the antibody response and the abnormal physiologic responses. The tissues of the animals at the termination of the experiment contained more water than is found in normal rabbits.

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PREDNISONE AND PREDNISOLONE IN THE TREATMENT OF ALLERGIC DISEASES

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THE value of steroid therapy in the management of allergic diseases has been definitely established. It is generally agreed that their use is not a substitute for adequate allergic diagnosis and therapy. These products are extremely helpful because they tide the patient over the period during which an allergic survey is carried out. They are also of therapeutic value in the symptomatic treatment of status asthmaticus, intractable bronchial asthma, severe nasal allergy, intractable serum sickness, recurrent urticaria, and atopic dermatitis. However, such therapy is accompanied by the occasional development of sodium and water retention, and therefore a low salt diet and the concomitant administration of potassium are necessary. Because of such reactions, some patients cannot tolerate these preparations. Furthermore, some patients do not respond clinically to even higher doses of cortisone and hydrocortisone. Two new steroids have been developed recently which promise to obviate the difficulties mentioned above. These are prednisolone (metacortandralone) and prednisone (metacortandracin or Meticorten). They have been synthesized by Schering and correspond roughly to hydrocortisone and cortisone, respectively. Their chemistry as well as evidence that their administration suppresses urinary ketosteroids and reduces circulating eosinophils is available in recent literature.[1, 2] Several preliminary reports have also appeared on the clinical use of these preparations in various conditions as well as in allergic disorders.[1-6] Most of these reports, however, deal only with the effectiveness of one of these products, namely, prednisone, in asthma. Detailed studies as to structure and biologic activity are also obtainable.[7]

PURPOSE

This paper concerns itself with an evaluation of the antiallergic potency of the two products referred to above. Some of the patients included in the series reported in this paper had received other steroids previously. An apportunity for comparison of therapeutic effect was thus afforded. An attempt was also made to determine the presence, extent, and nature of side reactions from the two drugs.

METHOD AND MATERIALS

Prednisone and later prednisolone were employed on two classes of allergic patients: those whose allergic condition had been carefully and completely

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studied by general medical and allergic methods, thus excluding complications such as heart disease, bronchiectasis, emphysema, pulmonary fibrosis, etc. The clinical condition of these patients was so severe or intractable that other accepted forms of symptomatic therapy was relatively ineffectual. The second group was composed of allergic individuals whose manifestations were so acute that it was necessary to offer them some therapeutic relief while an allergic investigation was carried out and allergic therapy was being instituted. None of the patients employed in this study presented the usual accepted contraindications to the administration of steroids (tuberculosis, peptic ulcer, etc.). Total eosinophil counts, urinalysis, blood pressure, and weight determinations were carried out at periodic intervals. Continued treatment extended over a period ranging from one week to four months. In other words, these findings are based on relatively short-term therapy. The ages of the patients varied from 2 to 74 years. Practically all other medication was discontinued during treatment with either of the two products. The first fourteen allergic patients treated with prednisone were placed on a restricted sodium diet and on potassium chloride. For this reason, this group is not included in the present report, although the therapeutic results were entirely comparable to those reported below. A total of ninety-six allergic patients selected from both private practice and local allergy clinics were used for the present study. None of the patients forming the basis of this study were restricted as to sodium intake or were given potassium chloride. The starting dose of prednisone or prednisolone ranged from 10 to 20 mg. every 8 hours, or a total of 30 to 60 mg. a day in the majority of cases. This dose was continued for three to six days, at the end of which time it was reduced to 10 mg. every 8 hours for a few days; then 5 mg. was given every 12 to 24 hours if the clinical response from treatment was satisfactory. There was only a slight decrease in dosage in children. Tables I-VI are self-explanatory. They indicate the sex and age of each patient; the duration of the disease; the extent or severity of symptoms marked in terms of +1 to +4, respectively; whether the patient has had the benefit of an allergic survey; the period of treatment with prednisone or prednisolone; and the total quantity of these drugs taken during the period of treatment. It should be pointed out that for the most part these patients, even those who have had an allergic survey, have not had the benefit of prolonged and adequate allergic therapy. In other words, there was an urgency about their symptoms which necessitated institution of steroid therapy. This therapy was not always continuous; in many instances, as improvement occurred, the drug was discontinued or reduced for a variable period of time. Some patients had more than one allergic condition. For example, a patient may have had active asthma as well as severe nasal allergy—so that this patient may appear in two tables—once in the table dealing with bronchial asthma and again in the table dealing with allergic rhinitis. Such duplication does not occur in more than eight or nine instances. Periodic follow-up included an appraisal of the patient's subjective response, physical examination, urinalysis, eosinophil counts, and information as to side reactions. In instances where there was an associated infection, antibiotics were also given.

TABLE I. BRONCHIAL ASTHMA

PATIENTS NO.	INITIALS	SEX	AGE	DURATION	ACTIVITY	ALLERGIC SURVEY COMPLETED	PERIOD TREATED (WEEKS)	TOTAL DOSE (MG.)	RESPONSE	INCREASE WT.	INCREASE B.P.	MOON FACE	ADD. THERAPY	REMARKS	ASSOC. DIAG.	SIDE REACTIONS
1	R. E.	F	51	12 Yr. (Mo.)	+3	x	16	3,600	+4	0	0	No	No		All. Rh.	2
2	S. J.	M	48	14	+4	x	4	900	+4	0	0	No	No		Sinusitis	None
3	K. K.	F	3½	2	+4	x	10	1,150	+2	0	0	No	No	Hay Fever		None
4	H. K.	M	32	12	+4	0	3	900	+2	0	0	No	No	Anxiety state		None
5	J. K.	M	47	15	+2	x	8	1,800	+4	0	0	No	No		H. F.	None
6	J. T.	F	32	5	+2	0	2	1,250	+3	0	0	No	No		All. Rh.	None
7	H. F.	F	66	15	+4	x	3	900	+4	0	0	No	No		H. F.	1, 2
8	M. F.	F	74	20	+3	0	3	850	+2	0	0	No	No	Complications	Emphysema	
9	R. D.	F	32	2	+4	0	4	1,350	+4	0	0	Yes	No	Discontinued	Emphysema	
10	Y. B.	F	38	10	+4	0	1	150	+4	0	0	No	Sed.			1, 2, 4
11	B. B.	M	11	8	+4	x	6	750	+3	0	0	No	No	Nose improved	H. F.	
12	J. C.	M	41	12	+4	x	4	450	+4	0	0	No	No	Encouraged		
13	H. T.	F	38	12½	+4	x	4	450	+4	0	0	No	No		All. Rh.	
14	C. T.	M	46	14	+4	x	3	425	+2	0	0	No	No	Antibiotics	Bronchiectasis	
15	G. S.	M	54	7	+3	x	3	400	+4	0	0	No	No		Rh. Heart	
16	E. S.	M	25	4	+2	x	5	925	+3	0	0	No	Sed.		All. Rh.	
17	H. R.	M	52	8	+2	0	4	1,325	+4	0	0	No	No	Discontinued	All. Rh.	2, 3
18	M. S.	M	50	12	+4	0	5	490	+4	0	0	No	No	Status		
19	L. R.	F	62	9	+4	0	3	435	+4	0	0	No	No	Status		
20	A. C.	M	28	18	+4	x	8	900	+3	0	0	No	No	Status		
21	L. I.	F	54	18	+3	x	8	1,250	+1	0	0	No	No	Anxiety		2, 3, 4
22	W. F.	M	51	15	+3	x	4	450	+2	0	0	No	No			
23	G. F.	M	31	5	+3	x	12	1,350	+4	0	0	No	No	Antibiotic	Urticaria	
24	C. E.	M	2	2	+3	x	3	600	+4	0	0	No	No		All. Rh.	
25	E. M.	F	2½	2	+4	0	4	450	+4	0	0	No	No		All. Rh.	
26	E. L.	F	4	2	+4	0	4	450	+3	0	0	No	No	Antibiotics	Sinusitis	
27	C. R.	F	5½	3	+4	x	3	340	+4	0	0	No	No		Sinusitis	
28	C. Y.	F	62	15	+4	0	2	450	+3	0	0	No	No		Sinusitis	
29	L. C.	F	68	12	+4	0	1	450	+3	0	0	No	No			
30	W. P.	M	52	8	+4	x	2	210	+4	0	0	No	No			1, 4
31	L. B.	M	42	7	+4	x	2	310	+4	0	0	No	No			
32	W. B.	M	38	6	+4	x	3	450	+4	0	0	No	No			
33	W. H.	M	44	8	+4	x	3	275	+4	0	0	No	No	Intermittent		

KEY TO TABLES:
All. Rh.=Allergic rhinitis.
H.F.=Hay fever.
Rh. Heart=Rheumatic heart disease.
Sed.=Sedation.
+4=Marked.
x=Yes.
0=None.

BRONCHIAL ASTHMA (TABLE I)

A total of thirty-three patients suffering from active bronchial asthma were treated. The age range was from 2 to 74 years. There were twenty-two men and eleven women. The duration of the asthmatic manifestations was from two to twenty years. Several patients had associated purulent sinusitis. Two patients had emphysema, one had bronchiectasis, one rheumatic heart disease without heart failure, and several presented evidence of associated allergic manifestations, such as allergic rhinitis, both seasonal and perennial. Urinalysis in every case was negative for sugar.

Several patients were hospitalized for status asthmaticus. These patients received prednisone, 20 mg. every 8 hours, and potassium iodide. Within three to four days, their condition improved considerably and at the end of one week they were both practically symptom-free on maintenance doses of prednisone. For the most part the dose of prednisone or prednisolone was as indicated above. After the condition improved, it became possible to taper off the dosage to a maintenance level of 5 mg. every twelve hours. Six patients in this series showed side reactions, making it necessary to discontinue prednisone. One of these patients developed severe epigastric distress and gas eructations, both of which were relieved by aluminum hydroxide, but the patient refused to resume therapy. The second patient complained of severe insomnia and abdominal cramps, dizziness, and vomiting, all of which symptoms disappeared after the prednisone was discontinued. Neither of these two patients showing side reactions presented roentgen-ray evidence of peptic ulcer. One asthmatic individual in this series received cortisone for four weeks, 250 mg. per day, without any appreciable effect. This young woman responded dramatically to prednisolone 60 mg. per day after five days. Another patient, a middle-aged asthmatic mill worker, gained 20 pounds in weight while on hydrocortisone. The change to prednisone was associated with diuresis and return to his normal weight. None of the patients showed glycosuria, elevation of blood pressure, cardiographic changes indicative of hypopotassemia, or evidence of water retention. One patient developed "moon face," necessitating discontinuance of the drug.

PERENNIAL ALLERGIC RHINITIS (TABLE II)

A total of twelve patients with perennial allergic rhinitis were treated. Most of these individuals had severe nasal allergy with complete and continued nasal obstruction, rhinorrhea and sneezing which did not respond quickly to the usual antiallergic therapy. These twelve patients were completely relieved of symptoms after receiving this new type of steroid medication. Eight patients received prednisone and four received prednisolone in a manner and dosage indicated above. Three patients had large nasal mucous polyps. Neither prednisone nor prednisolone caused these polyps to disappear, although the nasal airway became open under treatment. One patient developed severe dizziness and abdominal cramps while on prednisolone, making it necessary to discontinue the drug. There was no increase in blood pressure. None of the patients retained water and gained weight. There were no reactions of "moon face" and the urine in every case was negative for sugar.

H.F.=Hay fever.
Rh. Heart=Rheumatic heart disease.
Sed.=Sedation.
+4=Marked.
0=None.

TABLE II. ALLERGIC RHINITIS

PATIENTS NO.	INITIALS	SEX	AGE	DURATION (MO.)	ACTIVITY	ALLERGIC SURVEY COMPLETED	PERIOD TREATED (WEEKS)	TOTAL DOSE (MG.)	RESPONSE	ADD. THERAPY	REMARKS	ASSOC. DIAG.	SIDE REACTIONS
1	R. M.	F	51	10	+3	0	2	240	+4	Local	Anxiety	Br. A. mild	No
2	A. B.	F	31	5	+4	0	5	900	+4	Local		Urticaria	No
3	M. A.	F	28	3	+4	x	3	450	+4	Local		Sinusitis	No
4	D. O.	F	32			x	6	875	+4	Local			No
5	H. F.	F	66	15	+4	x	3	900	+4	Local		Br. A.	1, 2
6	E. S.	M	25	4	+4	x	5	925	+4	Sed.		Br. A.	No
7	M. S.	M	34	2	+4	x	4	450	+4	Local		Polyps	No
8	R. L.	F	38	5	+4	x	6	300	+4	Local		Polyps	No
9	A. C.	F	29	6	+4	x	5	450	+4	Local			No
10	E. W.	F	55	9	+4	x	3	275	+4	Local		Polyps	No
11	S. T.	F	46	2	+4	0	4	300	+4	Local			No
12	F. R.	M	40	4	+4	0	2	160	+4	Local			No

See Key to Tables following Table I.
Br. A. = Bronchial asthma.

TABLE III. SEASONAL HAY FEVER

PATIENTS NO.	INITIALS	SEX	AGE	DURATION	ACTIVITY	ALLERGIC SURVEY COMPLETED	PERIOD TREATED (WEEKS)	TOTAL DOSE (MG.)	RESPONSE	ASSOC. DIAGNOSIS	SIDE REACTIONS
				(Yr.)							
1	J. H.	M	12	10	+3	x	2	260	+4	Br. asthma. All. rhinitis	None
2	H. G.	M	52	11	+3	x	3	335	+4		None
				(Mo.)							
3	J. J.	F	23	5	+4	x	2	145	+4		None
4	E. F.	M	61	29	+4	x	2	250	+4	Br. asthma	None
5	R. D.	F	44	46	+4	x	3	315	+4		None
6	P. D.	F	51	28	+4	x	2	180	+4		None
7	M. C.	M	28	18	+4	x	3	275	+4		None
8	G. C.	F	28	49	+3	x	3	400	+4		None
9	F. C.	M	10	53	+4	0	2	175	+4	Colitis	1, 3
10	C. W.	F	50	64	+4	0	2	150	+4	Pollen asthma	None
11	A. T.	M	51	12	+4	x	1	125	+4		None

See Key to Tables following Table I.

SEASONAL HAY FEVER (TABLE III)

Eleven patients were treated during the 1955 grass hay fever season. While all but two of them had been studied by allergic means, none had preseasonal pollen therapy and no attempt was made to administer coseasonal treatment. This was done in order not to interfere with an evaluation of therapeutic value of these steroids. No additional therapy was given. For the most part, these patients were very uncomfortable before treatment was instituted. Prednisone or prednisolone was given intermittently depending on the occurrence of symptoms for the total number of days indicated on Table III. There was no increase in weight or blood pressure. No patient developed "moon face." The urine in every case gave a negative sugar reaction. One patient in this series developed abdominal cramps and diarrhea. This patient had a pre-existing history of mucous colitis. None of the patients showed an increase in weight. The results are indicated in the accompanying table. All of the patients were completely relieved of symptoms.

ATOPIC DERMATITIS (TABLE IV)

Eighteen patients suffering with this condition were included in this evaluation. There were several children. Eight patients received prednisone and ten were given prednisolone. There was no increase in weight or blood pressure. Urinalysis was negative. All of the patients had an atopic background. Many had an associated history of bronchial asthma, hay fever, or perennial nasal allergy. Two children were sufficiently ill to justify hospitalization. One of the adults had been given cortisone, 200 mg. per day for three weeks, with some improvement. Cortisone had to be discontinued, however, because the patient developed water retention and gained 15 pounds in weight. Concomitant therapy such as avoidance of soap, and various types of local therapy had been employed before with very little relief. Most of these patients were almost completely relieved at the end of one to three weeks of prednisone or prednisolone therapy. Two patients had severe side reactions. Three children showed a quick return of the eczema upon discontinuing treatment. Several patients showed only a slight improvement; these were patients with advanced secondary skin changes. One child had an associated nummular eczema and the response in this patient was equivocal. The average maintenance dose was 10 to 20 mg. per day. There was additional local therapy in every case.

URTICARIA AND ANGIOEDEMA (TABLE V)

A total of twelve patients, suffering with intractable recurrent urticaria and angioedema, received the drugs; two were given prednisolone and ten received prednisone. There was no additional therapy. One patient developed severe urticaria as part of a serum disease type of reaction following administration of penicillin. Another patient had an urticarial reaction to sulfonamides. The response in all of these patients were highly satisfactory at the end of three to five days of therapy. There was no increase in weight or blood pressure. "Moon face" did not occur. Urine was negative for sugar. Most of the patients were started on 45 mg. of the drugs daily. The dose was tapered off gradually.

TABLE IV. ATOPIC DERMATITIS

PATIENTS NO.	PATIENTS INITIALS	SEX	AGE	DURATION (MO.)	ACTIVITY	ALLERGIC SURVEY COMPLETED	PERIOD TREATED (WK.)	TOTAL DOSE (MG.)	RESPONSE	MOON FACE	REMARKS	ASSOC. DIAGNOSIS	SIDE REACTIONS
1	A. W.	F	21	21	+3	x	12	1,350	+2	No	Anxiety	Br. asthma	None
2	R. H.	F	26	8	+3	x	2	900	+3	No	Infection	Br. A., H.F.	1, 2
3	S. L.	F	17	5	+2	x	3	450	+4	No	Discontinued	Br. A., H.F.	None
4	W. B.	M	43	2 (Yr.)	+3	0	8	900	+4	No	Encouraged		None
5	P. H.	M	37	13 (Mo.)	+3	0	3	320	+2	No			None
6	E. S.	F	17	10	+3	0	10 (Mo.)	1,350	+2	No			None
7	R. D.	M	13	12	+2	x	4 (Wk.)	1,980	+3	No	Infection		None
8	P. M.	F	11	6	+2	0	6	875	+4	No		All. Rh.	3
9	L. C.	M	2	1½		0	4	450	+4	No		All. Rh.	None
10	G. C.	M	4	4		0	8	900	+3	No		All. Conjunctivitis	None
11	J. B.	M	3	2		0	8	900	+1	No	Nummular eczema		None
12	B. A.	F	21	5		x	4	450	+3	Yes	Had cortisone previously	Br. A.	None
13	C. S.	F	5	2		0	5	275	+3	No		Br. A.	None
14	W. S.	F	6	3		0	8	675	+3	No		Br. A.	None
15	H. R.	F	29	5		x	16	1,350	+4	Yes			None
16	E. S.	F	18	10		x	4	450	+2	No			None
17	M. L.	F	19	7		x	3	350	+4	No			None
18	W. D.	M	29	10		x	12	1,200	+3	No			None

See Key to Tables following Table I.

TABLE V. URTICARIA AND ANGIOEDEMA

PATIENTS NO.	PATIENTS INITIALS	SEX	AGE	DURATION (MO.)	ACTIVITY	ALLERGIC SURVEY COMPLETED	PERIOD TREATED (WEEKS)	TOTAL DOSE (MG.)	RESPONSE	REMARKS	ASSOC. DIAGNOSIS	SIDE REACTIONS
1	C. D.	M	34	6	+4	0	16	1,400	+4		Allergic rhinitis	None
2	H. J.	M	39	1	+4	0	12	1,000	+4			None
3	G. S.	F	43	2	+4	0	3	275	+4			None
4	N. G.	F	19	2	+4	x	3	495	+4			None
5	C. K.	F	26	6	+4	x	1	200	+4		Serum sickness	None
6	C. A.	M	32	1	+4	0	2	410	+4		Drug allergy	None
7	H. C.	M	39	4	+4	0	12	780	+4			None
8	W. B.	M	43	2	+4	x	10	600	+4			None
9	S. C.	M	44	1	+4	0	2	190	+4			1
10	J. G.	M	33	4	+4	x	3	215	+4	Cortisone		None
11	J. H.	M	44	3	+4	x	2	130	+4	Leather		None
12	V. A.	F	22	1	+4	0	1	75	+4	Poison ivy		1, 4

See Key to Tables following Table I.

TABLE VI. CONTACT DERMATITIS

PATIENTS NO.	PATIENTS INITIALS	SEX	AGE	DURATION (MO.)	ACTIVITY	ALLERGIC SURVEY COMPLETED	PERIOD TREATED (WEEKS)	TOTAL DOSE (MG.)	RESPONSE	REMARKS	ASSOC. DIAGNOSIS
1	A. C.	F	41	1	+4	x	1½	250	+4		Occupational
2	J. G.	M	34	2	+4	x	1	190	+2	Recurs	Plant weeds
3	J. L.	F	28	1	+4	x	2	190	+4		Poison ivy
4	J. H.	M	43	½	+4	x	2½	200	+4		Occupational
5	A. F.	M	44	2	+4	x	3	185	+4		Poison ivy
6	S. P.	F	30	2	+4	x	1	275	+2		Seborrhea
7	S. T.	F	35	1	+4	x	2	490	+4		
8	W. B.	F	39	1	+4	x	3	460	+4		
9	S. L.	M	18	½	+4	x	3	370	+4		
10	G. S.	M	22	1	+4	x	3½	475	+3		

See Key to Tables following Table I.

Smaller maintenance doses were needed, averaging about 5 mg. twice a day. One patient developed nervousness and dizzness following prednisolone; another patient developed diarrhea and cramps while taking prednisone. The drugs were discontinued in both of these patients although there was a serious doubt as to whether the symptoms in the first one were due to prednisolone.

CONTACT DERMATITIS (TABLE VI)

Ten patients with severe allergic type of contact dermatitis were treated. Several patients had poison ivy dermatitis. Within a few days after therapy was begun, with 45 mg. of prednisone per day, the condition improved sufficiently so that the dose could be reduced, and finally the drug could be discontinued at the end of ten to twelve days. Additional local therapy was given. There were no side reactions in this group.

COMMENT

There is very little doubt that both prednisone and prednisolone are highly effective antiallergic products. Almost without exception, patients suffering from acute allergic manifestations were practically or completely relieved within five to twelve days of such therapy. Certainly these agents compare most favorably with cortisone and hydrocortisone. There seemed to be no difference in the efficacy of prednisone and prednisolone. Both appear to be about three to five times as potent as cortisone, and two to four times as potent as hydrocortisone, respectively. This was a short-term study and therefore offers no information as to the effects of long-term therapy. It is, however, reasonable to assume that, as in the case of treatment with other steroids, dosage should be tapered off gradually in order to prevent an adrenal crisis. This study also indicates that while the water retention characteristic of cortisone and hydrocortisone is avoided by the use of these new steroids, yet "the moon face" reaction is not avoided. While on the subject of side reactions which might develop from the administration of prednisone, I would like to cite a case which was brought to my attention.* A 44-year-old woman suffering from rheumatoid arthritis received about 45 mg. of prednisone daily for three months. While in the hospital she developed severe abdominal pain, fever, and diarrhea, which led to a fatal termination. Post-mortem examination revealed organized thrombi in the coronary arteries, mesenteric vessels, and right colic artery, with perforation of the transverse colon. These findings may be interpreted to be caused by, or associated with, the administration of prednisone, and may be due to the effect of the drug on blood coagulability.[8] It should be pointed out, however, that the maintenance dose of prednisone in our series of patients is much less, approximating 10 to 15 mg. per day.

SUMMARY AND CONCLUSIONS

Ninety-six patients with active and sometimes uncontrollable allergic manifestations were treated. About two-thirds of these patients received prednisolone and the remainder were treated with prednisone. There were thirty-three

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instances of bronchial asthma and twelve of perennial nasal allergy. There were eleven instances of seasonal hay fever and eighteen of atopic dermatitis. In addition, twelve patients with urticaria and ten patients with allergic contact dermatitis are included. Treatment in no instance was carried out over a period longer than four months. The symptomatic improvement was almost universally satisfactory. A total of thirteen out of ninety-six patients showed undesirable side effects after four to twelve days of therapy. Treatment with these drugs was discontinued in these patients. There was no instance of elevation of blood pressure, peripheral edema, or clinical evidence by electrocardiogram studies of hypopotassemia. Aluminum hydroxide relieved the gastric symptoms in two patients. Two patients showed evidence of water retention while on cortisone; they began to lose weight after cortisone was discontinued and prednisone was administered. One or two patients who showed very little reponse to adequate cortisone therapy improved when given 45 mg. of prednisone per day for five days, Three patients showed "moon face" reaction. In one of these instances the patient had received cortisone previously. One instance is cited in which fatal perforation of the colon occurred after three months of therapy with fairly large doses of prednisone.

Prednisone and prednisolone are steroids valuable in the symptomatic treatment of active allergic conditions. Their administration does not necessitate the concomitant administration of potassium chloride and a low salt diet. Their use is not intended as a substitute for adequate allergic diagnosis and allergic management.

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LIBERATION AND DEPLETION OF HISTAMINE FROM HUMAN SKIN

Effect of an Antihistamine and Hydrocortisone on the Activity of the Histamine Liberator, Compound 48/80

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IN A previous paper, we have reported[1] that compound 48/80, a condensation product of p-methoxyphenylethyl-methylamine with formaldehyde, is a histamine liberator in man. Intradermal injections in man produced wheal and flare reactions. Repeated injections diminished the local histamine supply, so that subsequent injections of this compound, or of a specific antigen, produced a diminished response or no response. Sites depleted with compound 48/80 or a specific antigen still reacted to intradermal injections of histamine, but to a lesser degree than in the uninjected sites or sites previously pretreated by injections of histamine. Evidence was thus present that, in addition to histamine depletion, a part of the refractoriness from the injection of a specific antigen or compound 48/80 may be due to an injury or a modification of the tissues.

The present article deals with an attempt to study the nature of this tissue change resulting from histamine liberators. The first question to which we desired an answer is: Is this tissue change due to the liberation of another chemical substance or is it due to the endogenous histamine itself, perhaps because of its intimate contact with the cell or because of injury of the cell as a result of the process of liberating its histamine? It was thought that the degree and nature of protection from local injection of an antihistamine or hydrocortisone might furnish a clue to the mechanism of this tissue effect.

PROCEDURE

Four nonallergic adults were chosen for these experiments. All injections were given intradermally on the back. Preliminary titrations were made with 0.01 c.c. intradermal injections of histamine and compound 48/80. Histamine, in terms of base, was used in concentrations of 1:33,000 to 1:8,100,000 in threefold serial dilutions. Compound 48/80 was assayed with dilutions of 1:33,000 to 1:2,700,000, also in threefold serial dilutions.

Five vertical series of eight sites each were injected daily as follows: Series 1 sites were injected with 0.05 c.c. of Pyribenzamine, 1:300,000, followed in ten minutes by injections of 0.05 c.c. of compound 48/80, 1:33,000. This was repeated for five consecutive days. On the sixth day four of the sites were

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tested with intradermal injections of 0.01 c.c. of histamine, ranging in concentration from 1:900,000 to 1:33,000. The other four sites were tested with 0.01 c.c. of similar concentrations of compound 48/80. Tests with histamine and compound 48/80 were made in alternating sites.

Series 2 sites were injected daily for five days with 0.05 c.c. of Pyribenzamine 1:300,000. The final tests with histamine and compound 48/80 were made on the sixth day, twenty-four hours after the last injection of Pyribenzamine, exactly as carried out in Series 1 sites.

TABLE I. CHALLENGE OF SITES WITH COMPOUND 48/80

48/80 DILUTIONS	CONTROL UNTREATED SITES	PRETREATMENT WITH: 48/80	PYRIBENZAMINE	PYRIBENZAMINE + 48/80	HYDROCORTISONE	HYDROCORTISONE + 48/80
			Subject 1			
1:2,700,000	0	–	–	–	–	–
1:9,000,000	0	0	0	0	0	0
1: 300,000	‡	0	+	0	0	0
1: 100,000	+++	0	++++	++	++	0
1: 33,000	++++	+	++++	++	++++	0
			Subject 2			
1:2,700,000	0	–	–	–	–	–
1: 900,000	‡	0	0	0	0	0
1: 300,000	++	0	++	0	0	0
1: 100,000	+++	0	+++	‡	‡	0
1: 33,000	++++	0	+++	+	++	0
			Subject 3			
1:2,700,000	0	–	–	–	–	–
1: 900,000	0	0	0	0	0	0
1: 300,000	0	0	0	0	0	0
1: 100,000	++	0	+	0	0	0
1: 33,000	+++	0	++	+	++	‡
			Subject 4			
1:2,700,000	+	–	–	–	–	–
1: 900,000	+	0	0	0	0	0
1: 300,000	++	+	++	‡	+	0
1: 100,000	+++	++	++++	++++	+	‡
1: 33,000	++++	++	–	–	++	++

Key to Symbols:
–, Not tested.
0, No reaction.
‡, Slight erythema.
+, Wheal—3 mm.
++, Wheal—6 mm.
+++, Wheal—10 mm.
++++, Wheal—13+ mm.

The sites in Series 3 were treated a day earlier than those in Series 1 with an intradermal injection of 0.05 c.c. of hydrocortisone acetate (2.5 mg./c.c.). On the following five days each site was injected with 0.05 c.c. of the hydrocortisone suspension, followed in ten minutes by an 0.05 c.c. injection of compound 48/80, 1:33,000. On the final day the sites were tested with histamine and compound 48/80. The sites in Series 5 were injected for six days with 0.05 c.c. of hydrocortisone and final tests were made as usual. Series 4 sites were injected daily for five days with 0.05 c.c. of compound 48/80,

1:33,000. On the sixth day the sites were tested with 0.01 c.c. amounts of dilutions of histamine and compound 48/80. On the final day of the experiment, previously uninjected skin was assayed again for response to histamine and compound 48/80 and compared with titrations at the beginning of the experiment.

RESULTS

The results are summarized in Tables I and II.

TABLE II. CHALLENGE OF TREATED SITES WITH HISTAMINE

HISTAMINE DILUTIONS	CONTROL UNTREATED SITES	PRETREATMENT WITH: 48/80	PYRIBENZAMINE	PYRIBENZAMINE + 48/80	HYDROCORTISONE	HYDROCORTISONE + 48/80
			Subject 1			
1:2,700,000	0	–	–	–	–	–
1: 900,000	‡	0	0	0	0	0
1: 300,000	+	0	+++	+	‡	0
1: 100,000	+++	++	++++	+++	+++	0
1: 33,000	++++	++	++++	++++	++++	+
			Subject 2			
1:2,700,000	0	0	0	0	+	0
1: 900,000	+	0	++	+	+	‡
1: 300,000	++	‡	+++	+++	++	‡
1: 100,000	+++	++	++++	++++	++	‡
1: 33,000	++++	–	–	–	–	–
			Subject 3			
1:2,700,000	–	–	–	–	–	–
1: 900,000	‡	0	‡	‡	0	0
1: 300,000	+	0	++	+	‡	0
1: 100,000	++	‡	++	+	+	0
1: 33,000	+++	+	+++	++	+	+
			Subject 4			
1:2,700,000	‡	0	+	0	0	0
1: 900,000	+	0	+	0	0	‡
1: 300,000	+	+	+	+	+	‡
1: 100,000	++	++	++	++	++	++
1: 33,000	+++	–	–	–	–	–

Key to Symbols:
–, Not tested.
0, No reaction.
‡, Slight erythema.
+, Wheal—3 mm.
++, Wheal—6 mm.
+++, Wheal—10 mm.
++++, Wheal—13+ mm.

Sites injected daily with compound 48/80 (Table I, column 2) show marked decrease in final response to 48/80 and a lesser decrease in response to histamine. These findings confirm those previously reported by us.

Sites tested with compound 48/80 after several daily injections of hydrocortisone (Table I, column 5) show a lesser reactivity to the histamine liberator. Similarly treated sites tested with histamine (Table II, column 5) appear to show a somewhat lesser reactivity than do untreated sites, but this diminution is not as marked as in the case of the challenge with compound 48/80. Sites

treated with Pyribenzamine alone (Tables I and II, columns 3) appear to have little, if any, influence on the final response to histamine or compound 48/80.

Sites treated daily with compound 48/80, preceded by injections of hydrocortisone (Table I, column 6), show a marked loss of reactivity on the final testing with compound 48/80. This reduction in reactivity is approximately equal to that obtained in the sites depleted with the histamine liberator without the prior injection of hydrocortisone. The histamine response in sites pretreated with compound 48/80 preceded by hydrocortisone (Table II, column 6) is about the same as that without the use of hydrocortisone.

Sites challenged with compound 48/80 after repeated injections of Pyribenzamine and compound 48/80 (Table I, column 4) show evidence of depletion, as indicated by a lesser response as compared to untreated sites. However, the response is greater than in the sites treated with compound 48/80 alone and much greater than when hydrocortisone was employed to protect the sites injected with compound 48/80. The histamine response of sites previously treated with Pyribenzamine and compound 48/80 (Table II, column 4) is greater than without the protective effect of Pyribenzamine and is about equal to that obtained in the untreated control sites.

DISCUSSION

In previous experiments three mechanisms were offered as possible explanations of the phenomenon of reduced histamine sensitivity of skin sites repeatedly treated with the histamine liberator, compound 48/80. One was mechanical trauma; this was ruled out by the lack of effect of injections of normal saline. A second was refractoriness from repeated vasodilatation. The lack of influence of repeated histamine injections on the final histamine response ruled out that possibility. The third possible mechanism was a tissue injury due to either a toxic substance other than histamine, the injurious nature of histamine from within the cell, or the destruction or injury of the cells in the process of the liberation of histamine from them.

The challenge with compound 48/80 of sites pretreated with Pyribenzamine and 48/80 showed some kind of protective effect from the Pyribenzamine. This protection presumably could be ascribed to one of the following mechanisms: (1) prevention of histamine release; (2) prevention of cellular injury from the released histamine or other mediator. The fact that the histamine challenge of sites pretreated with Pyribenzamine and compound 48/80 showed greater response than sites treated with the histamine liberator alone is evidence in favor of the protective action of the antihistamine on the cell. This is supported further by the demonstration that repeated injections of Pyribenzamine alone had no influence on the final challenge with compound 48/80 or histamine. Apparently this protective or competing action is greater than the previously described histamine-liberating action of Pyribenzamine.[2]

The challenge with compound 48/80 of sites previously treated with hydrocortisone and compared with sites untreated with hydrocortisone showed a lesser reactivity of the treated sites. This could be explained by any of the

following possibilities: (1) hydrocortisone also releases and depletes histamines; (2) hydrocortisone produces cell injury; (3) hydrocortisone diminishes capillary permeability or modifies the tissue response in some other manner. The lack of significant difference in the histamine response of sites treated with hydrocortisone and those untreated would speak against a cell injury effect. The combined findings may be interpreted as indicating that the hydrocortisone effect described here is primarily one of histamine depletion.

SUMMARY

1. The diminished response to histamine in skin sites previously treated with the histamine liberator, compound 48/80, was confirmed.

2. Pyribenzamine injected in the sites shortly before each injection of compound 48/80 did not prevent depletion of histamine by the latter.

3. Pretreatment with Pyribenzamine prevented that degree of refractoriness apparently resulting from tissue damage by the histamine liberator.

4. These findings suggest that the refractoriness of the tissue to histamine response subsequent to treatment with the histamine liberator owes its action to the damaging effects of released histamine in intimate contact with the cell.

5. It is suggestive, but not conclusive, that the local protective action of hydrocortisone in these experiments is due mainly to histamine depletion.

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STUDIES ON RAGWEED POLLEN. I. PRELIMINARY FRACTIONATION OF RAGWEED POLLEN

Characterization and Allergenicity of the Isolated Fractions

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INTRODUCTION

THE study of the chemical and immunologic properties of ragweed pollen was begun as early as 1831, when Elliotson[1] suggested ragweed as a possible causative agent of hay fever, an assumption that was later fully substantiated by the studies of Blackley.[2] Since then, numerous attempts have been made to isolate and characterize the active substances present in ragweed pollen, but with little success.

The results of chemical fractionation of ragweed pollen extract by applying different precipitating agents, such as ammonium sulfate,[6-9, 13, 16, 24, 36] alcohol,[3-5, 15, 19, 37, 48] acetone,[31] lead acetate,[21, 36] acids,[10, 11, 25, 26] and alkali,[7, 15, 21] have suggested allergenicity to be due to a carbohydrate or a protein constituent, or to both.

Using Tiselius electrophoresis, Newell[38] and Abramson and his associates[23] demonstrated the complex nature of ragweed pollen extract by showing that it can be resolved into a number of electrophoretically distinct components. Abramson and associates[23] using this method, were able to separate one of the faster migrating, pigmented components from the main immobile, colorless fraction. Allergenic activity was elicited by both fractions.

Abramson and associates[23] also determined a sedimentation constant of 1.5 for the major unpigmented, immobile material and calculated a molecular weight of 5,000 for this material. Sanigar[47] calculated a sedimentation constant of 1.0 for his ragweed extract, and concluded that the molecular weight could not exceed 17,000.

Stevens and his co-workers[40] determined a sedimentation constant of 1.3 for the nondiffusible fraction of their ragweed extract and calculated a molecular weight of 17,800 for this material. In a second study,[39] using free electrophoresis and melting point determinations, they showed that one of the pigments in ragweed pollen was isoquercitrin.

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Loveless and her associates[29] in 1951, using standard Visking tubing, were able to obtain a separation between the diffusible and nondiffusible components in ragweed pollen extract. Both fractions exhibited allergenic reactivity, but the nondiffusible material was the more active. Free electrophoresis revealed the presence of an essentially immobile, colorless component in both fractions.

Alexander and his associates[30, 31] fractionated, by paper chromatography, the dialysate of the ragweed pollen extract into five different components. Four of these stained for peptides and one for carbohydrate. The nondialyzable fraction did not stain for either component; but, after undergoing acid hydrolysis, this latter material stained with ninhydrin. These facts would indicate that the nondialyzable material was proteinous in nature. Allergenic activity was detected in all the fractions.

The results of the fractionation of ragweed pollen by paper chromatography by Bernstein and his co-workers[41] and Perlman[42] also emphasized the complex composition of ragweed pollen and the presence of numerous allergenically active constituents.

Bookman and Wax,[43] using ion exchange resins, demonstrated that the allergenicity of ragweed pollen fractions is totally unrelated to their respective nitrogen contents.

Munoz and Becker,[28] applying the Oudin "gel technique,"[33, 34] clearly established the existence of at least five different antigens in ragweed pollen extracts. Wodehouse[32] was able to demonstrate seven or eight precipitin bands by the Ouchterlony "gel technique,"[34, 35] each band representing a different antigen in the pollen extract.

In 1954 Abramson and his associates[44] claimed to have prepared a "purified" ragweed extract by treatment of defatted ragweed pollen with alcohol prior to extraction with water. This latter extract, designated by them MLL (methanol leached and lyophilized), exhibited only two peaks on free electrophoresis and was therefore considered to be a "purified" extract.

Since it was our intention to study the immunologic and physicochemical properties of the various "pure" ragweed components, we chose for our initial study the "purified" fraction as prepared by Abramson and associates.[44] However, as shown in this report, this "purified" fraction proved to be a complex mixture of materials.

METHODS AND MATERIALS

Low ragweed pollen (*Ambrosia artemisiaefolia*) was defatted with diethyl ether in a Soxhlet extraction apparatus for three days. MLL was prepared by the method of Abramson and associates[44] in the following manner:

Five grams of the defatted pollen was leached with 50 c.c. aliquots of 90 per cent CH_3OH and the extracting medium was changed frequently until it was completely colorless. This alcohol-soluble fraction next was distilled under suction at 50 to 60° C. until alcohol could no longer be detected, and

then it was lyophilized. This fraction is referred to as ROH SOL. The residue from the alcohol extraction was dried and extracted with water for seventy-two hours. This extract was immediately lyophilized and constitutes MLL. A portion of the insoluble residue was then extracted continually with water for twelve days longer, the extracting fluid being replaced every two days, and the former was lyophilized. This preparation is referred to as MLL INSOL. All extractions with alcohol and water were performed in a cold room at 3 to 5° C. The water used in all extractions, except where specified, was distilled water.

To determine the specific effect, if any, of alcohol on the composition and allergenicity of ragweed pollen, MLL was compared with a water extract of ragweed pollen which had not undergone preliminary alcohol leaching. The water extract of pollen was prepared by extracting 10 grams of ether-defatted ragweed in 100 c.c. of water for seventy-two hours. The mixture was then centrifuged at 20,000 r.p.m. for twenty minutes in a Spinco Model L preparative ultracentrifuge. The yellow-colored supernatant was slightly turbid, this opalescence being attributed to some lipoidal material still present in ragweed pollen after the extensive defatting procedure. The solution was then lyophilized, redissolved in a small volume of water (20 c.c.), and recentrifuged at 20,000 r.p.m. for twenty minutes. A white material rose to the surface of the liquid, and the clear, yellow-colored supernatant was drawn off with a syringe provided with a long needle and lyophilized. This material is referred to as WSR (water-soluble ragweed). The insoluble residue left after the seventy-two hours' extraction of the defatted pollen with water was further extracted continually for sixteen days with water, the extracting fluid being changed every two days. The insoluble residue which remained was lyophilized and is referred to as WSR INSOL.

Both WSR and MLL were dialyzed through Visking tubing against water, the ratio of the volume of the solution within the Visking tubing to that outside being 1:5. In each case, the water solution of the fraction was first dialyzed for thirty-six hours, and the dialysate was subsequently lyophilized. This fraction is termed *dialysate*. The residue remaining within the sac was then dialyzed continually for ten days against running tap water to ensure complete removal of dialyzable material. It was then lyophilized and designated as *dialyzed residue*.

To determine whether allergenic reactivity was still associated with the MLL INSOL, a portion of this material was extracted with 0.05 N NaOH for seventy-two hours. The insoluble residue was then lyophilized, and is referred to as NaOH INSOL. The yellow-colored supernatant was then acidified to pH 6.0 with 0.1 N HCl, at which point a white, fluffy precipitate separated out. The supernatant was separated from this fine precipitate by centrifugation at 25,000 r.p.m. for twenty minutes, and is referred to as pH 6 SOL. The precipitate is referred to as NaOH SOL. Both extracts were subsequently lyophilized.

Fig. 1 shows a flow sheet of the complete extraction procedure of the various fractions from ragweed pollen.

The extracts were compared by the following four methods:

I. *Free Electrophoresis.*—The three fractions—MLL, WSR, and ROH SOL—were analyzed by free electrophoresis.

The Spinco Tiselius apparatus was used throughout the present study to determine the electrophoretic pattern of each fraction. The solutions for electrophoresis were prepared by dissolving the fractions in the following buffers:

(a) Veronal buffer, pH 8.6; ionic strength 0.1
(b) Borate buffer, pH 8.6; ionic strength 0.1
(c) Phosphate buffer, pH 7.8; total ionic strength 0.17 (contribution of NaCl 0.15)
(d) Glycine buffer, pH 10.6; total ionic strength 0.17 (contribution of NaCl 0.15)
(e) Glycine buffer, pH 3.5; total ionic strength 0.17 (contribution of NaCl 0.15)

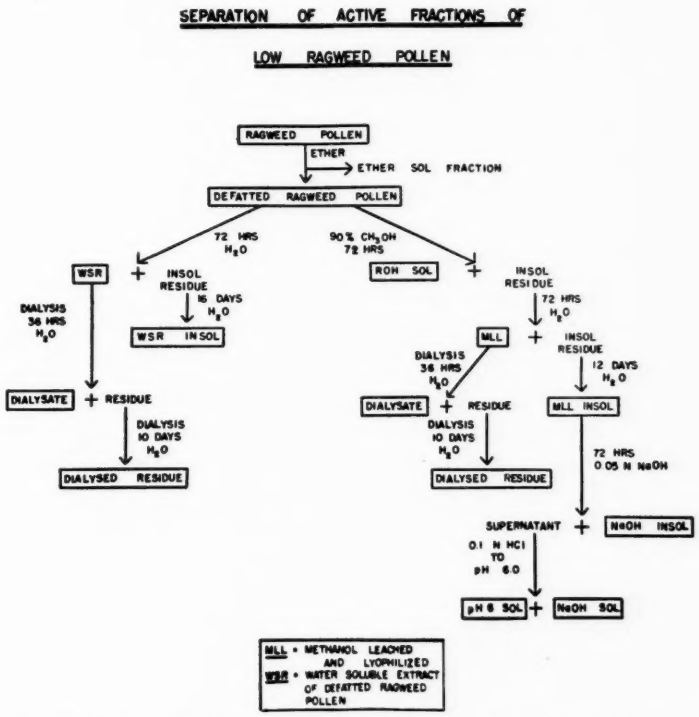

Fig. 1.—Flow sheet of the extractions of fractions from low ragweed pollen.

Each of the fractions was found to contain both diffusible and nondiffusible components on dialysis through Visking tubing. Therefore, when each fraction was analyzed by electrophoresis for all its constituents, initial dial-

ysis against the respective buffer was dispensed with. To minimize anomalies in the electrophoretic experiments, the conductivities of the buffer and of the respective extract-buffer solution were equalized prior to each run. The pH of the extract buffer solution was raised or lowered to that of the buffer by the addition of either 0.1 N NaOH or 0.1 N HCl; the conductivities were then equalized by adding solid NaCl to the buffer. These steps were necessary since, in all cases, the solution of any particular fraction in the buffer used had a higher conductivity than, as well as a different pH from, the corresponding buffer.

II. *Paper Electrophoresis.*—Whatman No. 1 filter paper was used throughout.

The aqueous solution of the material to be separated by paper electrophoresis was applied at the center of the strip of filter paper as a narrow band about 30 to 35 mm. long. The filter paper was previously immersed in the buffer and semidried between sheets of filter paper. The paper was then layered onto a horizontal Plexiglas plate* which was used as the supporting surface. This, in turn, was placed onto the buffer vessels, the two extremities of the paper dipping into the buffer to a depth of about one inch. The buffer vessels had previously been filled to an equal level, which is essential to prevent chromatographic effects from disturbing the separation pattern of the material under observation. For similar reasons, the plate supporting the filter paper strips was leveled both longitudinally and vertically.

Heating, caused by passage of current through the paper strips, results in some evaporation of water from the filter papers saturated with buffer. To minimize evaporation from the paper during electrophoresis, a glass plate supported by a foam rubber frame was placed onto the papers, forming a closed system. A sheet of Whatman No. 3 MM. filter paper was placed between the glass plate and the foam rubber frame. Its function was to absorb any water vapor which might condense on the glass plate during electrophoresis. Application of high voltages (above 170 volts) results in more evaporation from the paper. This, in turn, causes absorption of more buffer into the paper strips with a concomitant chromatographic displacement of the electrophoretically separated bands toward the center of the paper. The effect of absorption of more buffer into the paper results in a further increase of the concentration of buffer salts in the filter papers. This, in turn, results in even more evaporation and in serious disturbances of the electrophoretic separation. These effects were minimized by immersion of the ends of the Whatman No. 3 MM. paper into the buffer vessels. Due to the lower resistance of this buffer-saturated filter paper, as compared to that of the Whatman No. 1 filter paper strips, more heat was dissipated from it. Consequently, the electrophoretic chamber was saturated with vapor emanating mainly from the What-

*The upper surface of the Plexiglas plate was provided with small, closely spaced, pyramidal projections, extending to a height of ⅛ inch above the surface of the plate. This arrangement prevented the paper from sagging and the formation of a surface liquid film between the paper and the Plexiglas. The dimensions of the Plexiglas plate were 16 inches long by 15 inches wide.

man No. 3 MM. paper. The buffer vessels were then connected by paper wicks with the electrode vessels, filled to equal levels with 20 per cent KCl solution, containing silver-silver chloride electrodes.* These electrodes were used in preference to the conventional platinum or carbon and steel electrodes to ensure constancy of pH during electrophoresis.

Preliminary experiments demonstrated that optimal separation of ragweed pollen extracts was obtained by applying a potential of 110 volts, the current varying from 20 to 40 milliamperes, depending on the buffer used. Electrophoresis was carried out at room temperature. The duration of a single electrophoretic experiment never exceeded fourteen hours, but varied with the fraction being analyzed.

Upon completion of the run, the papers were dried quickly at 120° C. and then stained for carbohydrate, protein, amino acids, and peptides.

The following staining reagents were used:

(a) 1 per cent ninhydrin solution in 95 per cent ethyl alcohol to detect amino acids and peptides[50]

(b) The Lemieux-Bauer reagent to detect carbohydrates[49]

(c) Amido-black (10 B) to stain for proteins[46]

For the sake of brevity, all bands revealed by ninhydrin staining are referred to as peptide bands.

Buffers used in this study were:

(a) Veronal buffer, pH 8.6; ionic strength 0.10

(b) Borate buffer, pH 8.6; ionic strength 0.10

(c) Phosphate buffer, pH 7.8; ionic strength 0.17 (contribution of NaCl 0.15)

(d) Citrate buffer, pH 4.56; ionic strength 0.10

Preliminary experiments indicated that borate buffer provided the resolution of a maximum number of components of WSR and MLL. Therefore, results obtained with this buffer are the only ones reported here.

III. *Ultracentrifugation.*—The Spinco Model E optical ultracentrifuge was used to determine the sedimentation constants of the various fractions. The experiments also served to disclose any heterogeneity of the materials with respect to molecular weights.†

The synthetic boundary cell, as devised by Pickels and his co-workers[45] was used instead of the standard cell. This cell allows for the formation of a sharp initial boundary between the solution under observation and the solvent at the start of the experiment. Formation of initial boundaries is essential in determining the sedimentation rate of small molecular weight materials, which otherwise would diffuse at such a rapid rate as to prevent

*The silver-silver chloride electrodes were prepared from silver strips ¼ inch wide and 1/32 inch thick.

†Assuming that diffusion coefficients and partial specific volumes are equal for all fractions.

the formation of a sedimenting boundary. The average rotor temperature was 20.5° C. The sedimentation constants were not recalculated for standard conditions.

All the fractions, with the exception of ROH SOL, were dissolved in 0.9 per cent NaCl. The ROH SOL fraction was dissolved in glycine buffer at pH 10.6 due to its low solubility in 0.9 per cent NaCl and in solutions of lower alkalinity.

IV. *Cutaneous Reactivity.*—All the fractions were made up to 1,000 units per cubic centimeter (1 unit = 10^{-5} mg. nitrogen). Nitrogen values were determined by a modification of the method of Lanni, Dillon, and Beard.[51] All the fractions were tested on ragweed-sensitive persons. The activity was determined by scratch test and reactions were read after twenty minutes. Reactions were tabulated as 0, 1+, 2+, 3+, and 4+, depending on the size and extent of the wheal and surrounding erythema.

RESULTS

Initial extraction with alcohol, followed by extraction with water, resulted in a lower yield of extractable material from defatted pollen as compared with the yield of material extracted with water only, as shown in Table I. The data in this table were arrived at by assuming that the lyophilized fractions were completely dehydrated.

TABLE I

FRACTION	PERCENTAGE OF WHOLE DEFATTED RAGWEED
WSR 16-day extraction	30
ROH soluble ragweed	12
MLL	6
pH 6 soluble ragweed	20
NaOH soluble ragweed	8
NaOH insoluble ragweed	52

*Free Electrophoresis.**—The three fractions examined by free electrophoresis (WSR, MLL, and ROH SOL), using different buffer systems, were all found to be electrophoretically heterogeneous. An essentially immobile peak was present in all the electrophoretic patterns of the fraction. At least three distinct peaks were identified in each pattern. The most striking electrophoretic separation occurred with WSR in glycine buffer at pH 3.5. At least five well-characterized, small peaks were seen migrating to the negative pole at a rapid rate. The results are illustrated in Figs. 2, 3, 4, and 5.

Due to the low solubility of ROH SOL in buffers of pH lower than 9, it was analyzed by free electrophoresis only in glycine buffer at pH 10.6 (Fig. 5).

*Paper Electrophoresis.**—By paper electrophoresis in borate buffer at pH 8.6, WSR and MLL were resolved into visible pigment bands, in addition to distinct bands revealed by staining with ninhydrin, amido-black, and the carbo-

*In Figs. 2 through 11 the arrows represent the initial boundaries and zones of application in experiments using free electrophoresis and paper electrophoresis, respectively.

hydrate reagents. It is evident from Figs. 6 and 7 that WSR and MLL are distinct materials, containing different numbers of electrophoretically separable fractions. WSR is comprised of four pigment bands, one protein band, one carbohydrate band, and one peptide band which migrated independently

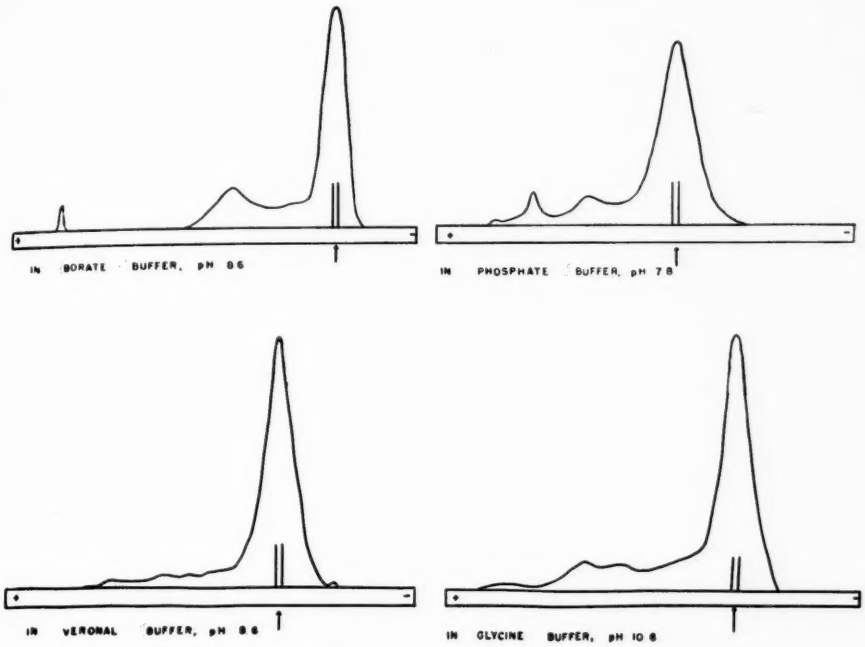

Fig. 2.—Free electrophoresis of the water extract of defatted low ragweed pollen (WSR).

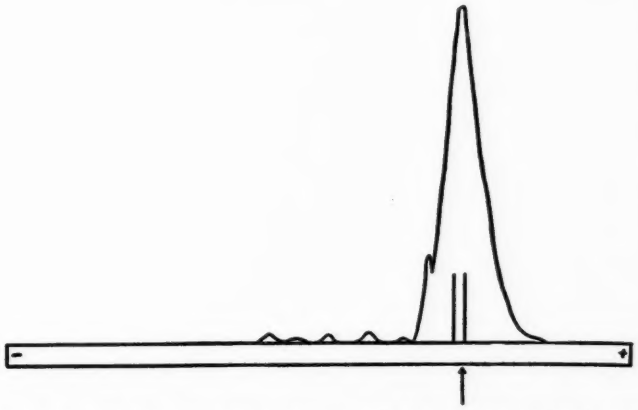

Fig. 3.—Free electrophoresis of the water extract of defatted low ragweed pollen (WSR) in glycine buffer, pH 3.5.

to the posititive pole; three peptide bands which migrated to the negative pole; and one diffuse peptide band which maintained itself at the center of the paper, but extended into both the cathodic and anodic regions of the paper. Since it extended more into the cathodic region, this slow migration

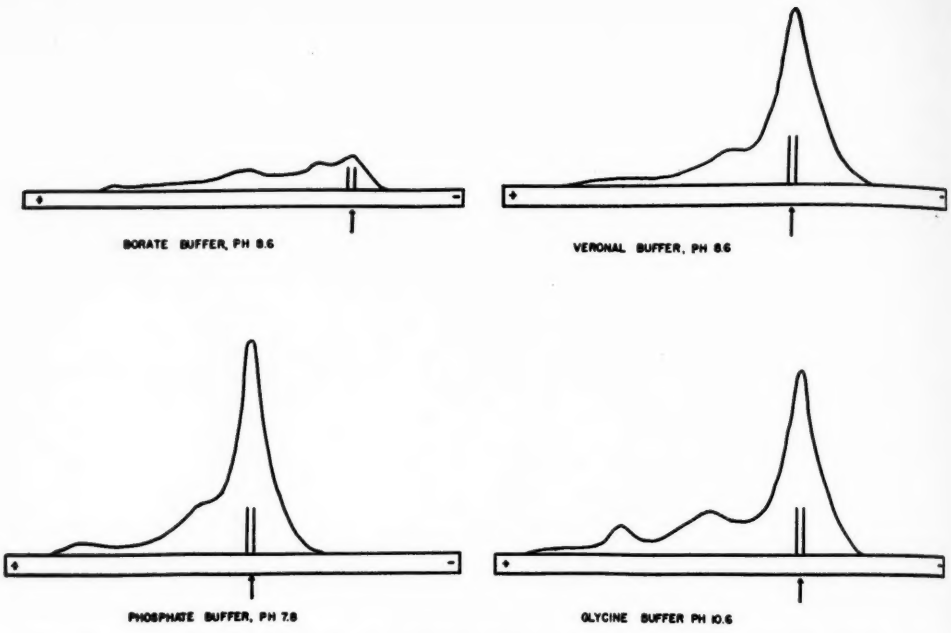

Fig. 4.—Free electrophoresis of the water extract of defatted low ragweed pollen after methanol extraction (MLL).

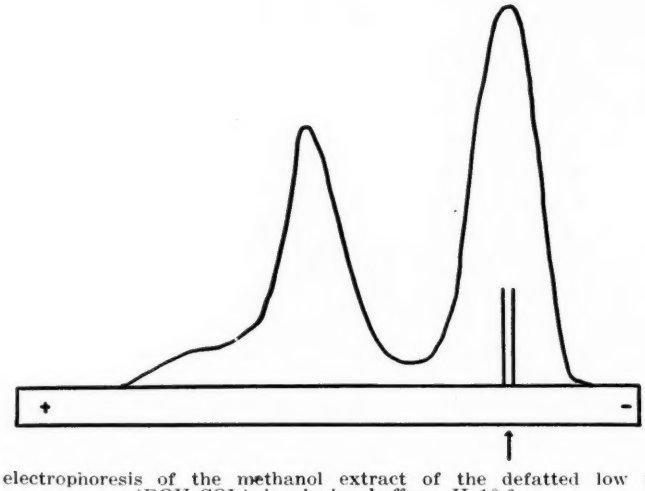

Fig. 5.—Free electrophoresis of the methanol extract of the defatted low ragweed pollen (ROH SOL) in glycine buffer, pH 10.6.

is considered to be due to electrophoresis rather than to diffusion. It ought to be mentioned that all the five peptide bands were detected only in the early stages (six hours) of electrophoresis of WSR in borate buffer at pH 8.6 (Fig. 6). In experiments of longer duration (twelve to fourteen hours), however, the two rapidly migrating cathodic bands were permitted to run off the paper in order to obtain an optimal resolution of the other components.

On the other hand, MLL in borate buffer at pH 8.6 (Fig. 7) separated into one diffuse pigment, one protein, one carbohydrate, and three peptide bands.

ROH SOL in borate buffer gave rise to three pigment bands (Fig. 8). No carbohydrate-stainable material could be detected in this fraction, while a faint ninhydrin band was noted at the cathodic side of the point of application.

The pH 6 SOL could not be resolved into more than one component by paper electrophoresis using Veronal and borate buffers. Electrophoretic separations with these two buffers at pH 8.6 resulted in identical patterns (Fig. 9).

A comparison of the electrophoretic patterns of the dialysate, dialyzed residue, and whole extract for WSR and MLL, respectively, as represented in Figs. 10 and 11, suggests that both WSR and MLL can be partially fractionated by dialysis through Visking membrane against water. The dialysate of WSR contained four pigment, three peptide, and one carbohydrate bands.

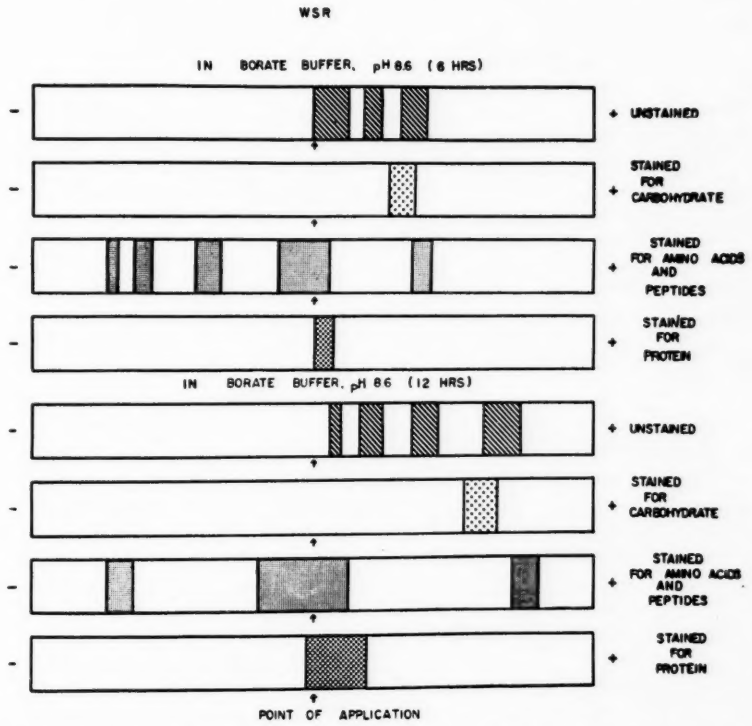

Fig. 6.—Paper electrophoresis of the water-soluble extract of defatted low ragweed pollen (WSR). The upper four diagrams represent the resolution of the constituents after six hours of electrophoresis. The lower four diagrams represent the separation of the components after twelve hours of electrophoresis.

The dialyzed residue consisted mainly of a protein-stainable material which migrated slowly to the anode. A slightly visible pigment band and a barely stainable carbohydrate band were seen migrating with it. In addition, a faint peptide band was detected which migrated slowly to the cathode.

Fig. 7.

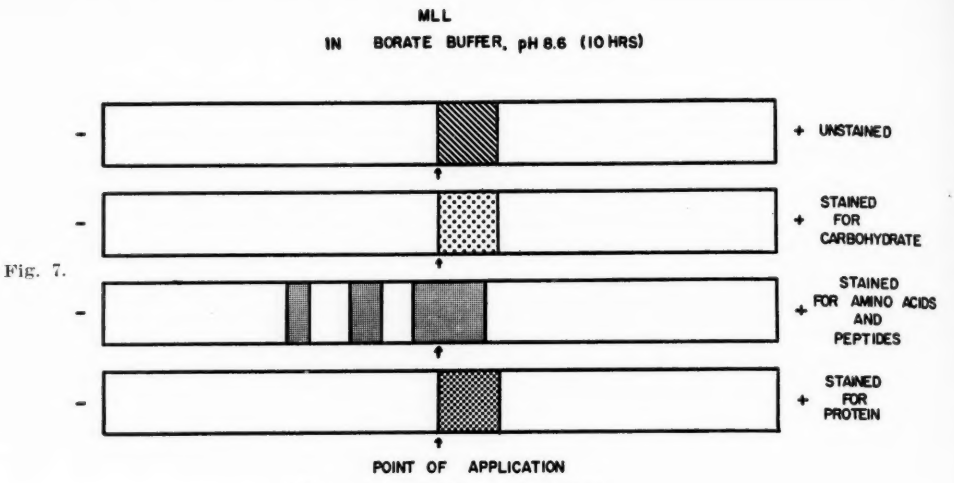
MLL
IN BORATE BUFFER, pH 8.6 (10 HRS)
- + UNSTAINED
- + STAINED FOR CARBOHYDRATE
- + STAINED FOR AMINO ACIDS AND PEPTIDES
- + STAINED FOR PROTEIN
POINT OF APPLICATION

Fig. 8.

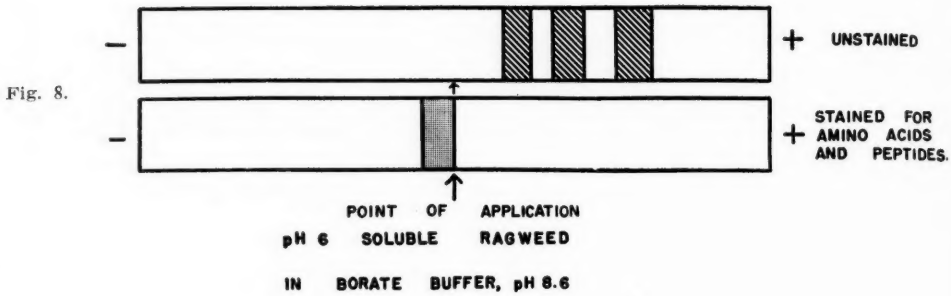

Fig. 9.

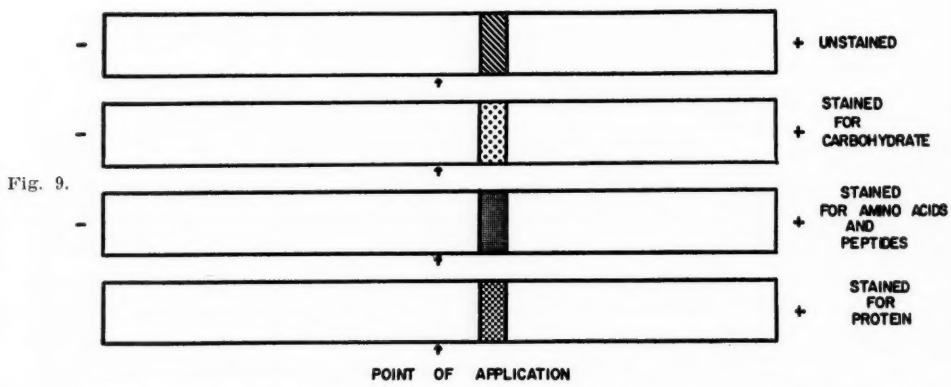
pH 6 SOLUBLE RAGWEED
IN BORATE BUFFER, pH 8.6
- + UNSTAINED
- + STAINED FOR CARBOHYDRATE
- + STAINED FOR AMINO ACIDS AND PEPTIDES
- + STAINED FOR PROTEIN
POINT OF APPLICATION

Fig. 7.—Paper electrophoresis of the water extract of defatted low ragweed pollen after methanol extraction (MLL).

Fig. 8.—Paper electrophoresis of the methanol extract of defatted low ragweed pollen (ROH SOL).

Fig. 9.—Paper electrophoresis of pH 6 soluble fraction. (For definition, see text.)

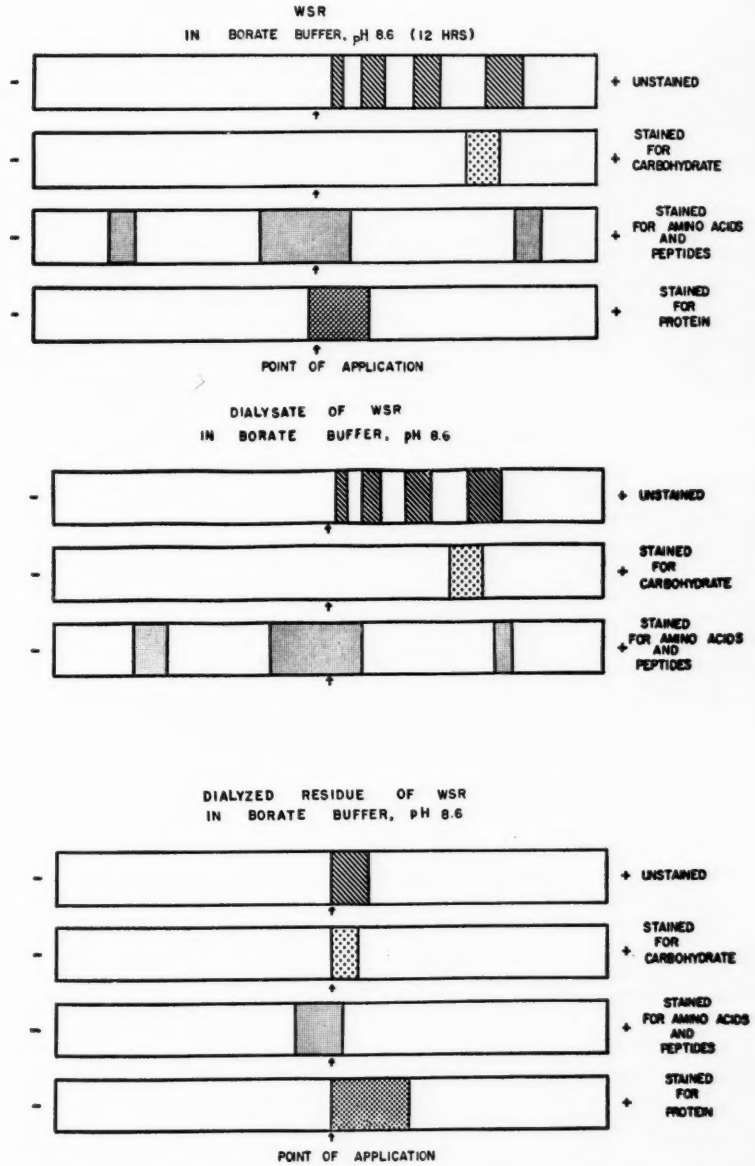

Fig. 10.—The upper four diagrams represent the electrophoretic separation of the components of WSR on paper. The middle three diagrams represent the separation of the constituents of the dialysate of WSR. The lower four diagrams represent the electrophoretic separation of the components of the dialyzed residue of WSR. Time for electrophoretic separation was twelve hours.

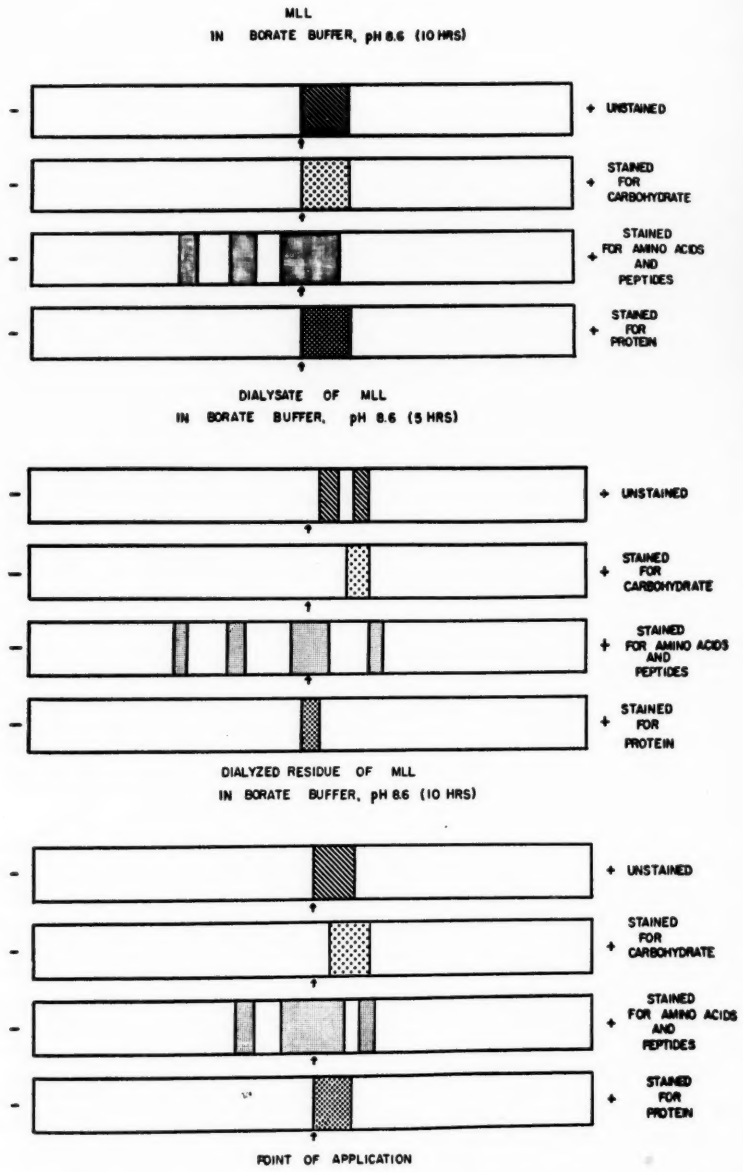

Fig. 11.—The upper four diagrams represent the electrophoretic separation of the components of MLL on paper after ten hours. The middle four diagrams represent the separation of the constituents of the dialysate of MLL after five hours. The lower four diagrams represent the components of the dialyzed residue of MLL resolved after ten hours.

The dialysate of MLL resolved itself into four distinct peptide bands, one carbohydrate band, and two pigment bands. In addition, a faint protein band was also detected. The four peptide bands represent bands migrating at a faster rate than those present in MLL. The dialyzed residue of MLL consisted of one protein, one pigment, one carbohydrate, and three peptide bands. The total number of distinguishable, electrophoretically separated bands in the dialysate and dialyzed residue was greater than the number detected in undialyzed MLL.

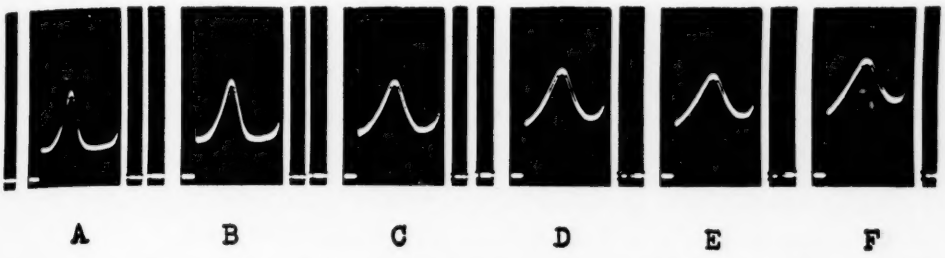

Fig. 12.—Sedimentation photographs of whole ragweed extract (WSR) taken at 59,780 r.p.m. Frame *A* represents the initial boundary in the synthetic boundary cell; frames *B*, *C*, *D*, *E*, and *F* represent photographs taken subsequently after twelve, eighteen, thirty-two, forty, and forty-eight minutes.

Ultracentrifugation.—Each extract gave rise to only one broad peak in the analytical ultracentrifuge. Fig. 12 is a photograph of a typical experiment. Sedimentation constants were determined for the majority of the fractions (Table II). The sedimentation constants were all rather low, varying from 0.45 to 2.54 Svedberg units. The WSR had a sedimentation constant of 0.92, while its dialysate and dialyzed residue had sedimentation constants of 0.61 and 2.54, respectively. The ROH SOL had the lowest sedimentation constant of 0.45, while that of the MLL was 1.34. A sedimentation constant of 1.17 was calculated for the pH 6 SOL.

TABLE II. ULTRACENTRIFUGAL ANALYSIS OF ACTIVE FRACTIONS SEPARATED FROM RAGWEED POLLEN*

FRACTION	SEDIMENTATION CONSTANT†
WSR	0.92
WSR—dialysate	0.61
WSR—dialyzed residue	2.54
ROH SOL ragweed‡	0.45
MLL	1.34
pH 6 SOL ragweed	1.17

*Performed in 0.9 per cent NaCl, except where indicated otherwise.
†Expressed in Svedberg units.
‡In glycine buffer pH 10.6.

Cutaneous Reactivity.—The results of cutaneous tests are shown in Table III. The WSR and ROH SOL fractions appeared to exhibit comparable skin reactivity, and the MLL and pH 6 SOL fractions only slightly less. The activity of the ROH SOL fraction diminished rapidly on standing, however, and completely disappeared after four weeks of storage in aqueous solution at

3 to 5° C. The activity of the other fractions also decreased with time, on storage in solution, but at a much more gradual rate. Loss of activity in other fractions could be demonstrated only after two to three months of storage in aqueous solution at 3 to 5° C.

In the present study, the ether-soluble fraction of ragweed pollen was not tested for skin reactivity. However, it was shown by Moore and his associates[14] to be active.

TABLE III. REACTIVITY OF FRACTIONS BY SCRATCH TEST

FRACTION*	SUBJECT TESTED								
	L. K.	M. G.	S. T.	A. M.	M. L.	D. K.	B. L.	B. K.	M. B.
pH 6 SOL	2+	±	0	4+	2+	±	±	3+	3+
NaOH SOL	±	0	0	0	0	0	0	0	0
NaOH INSOL	0	0	0	0	0	±	0	0	0
WSR	4+	2+	4+	4+	2+	2+	±	4+	–
WSR INSOL extracted with water—5 days	3+	0	±	4+	1+	±	–	3+	–
WSR INSOL extracted with water—14 days	–	–	–	–	2+	–	±	3+	4+
ROH SOL	4+	0	4+	0	0	±	–	0	0
MLL	4+	–	–	4+	3+	2+	–	4+	4+
MLL INSOL	±	0	0	0	1+	±	–	0	–

*Each made up of 1,000 units per cubic centimeter (1 unit = 10^{-5} mg. nitrogen).

The WSR INSOL, even after sixteen days of continuous extraction with water, still manifested positive cutaneous reactivity. MLL INSOL was not active when tested by scratch. However, the pH 6 SOL extracted from this seemingly inactive ragweed pollen residue was quite active. The NaOH INSOL, as well as the NaOH SOL, displayed essentially no whealing activity.

DISCUSSION

Free electrophoresis demonstrated that none of the preparations was homogeneous. A stationary component was distinguished in each of the electrophoretic experiments of the MLL, WSR, and ROH SOL fractions. One possible interpretation of these results is that ragweed pollen extract might contain a component having no ionizable groups, for it does not migrate in either acid or alkaline buffers. One type of substance which might behave in this manner could be a closed-ring polypeptide devoid of free amino or carboxyl groups. The large, diffuse stationary peak may also represent the contribution of salt boundaries.

Paper electrophoresis permits the fractionation of a complex solution of materials without preliminary adjustment of its pH and conductivity to those of the buffer. It proved to be a far more effective tool than free electrophoresis in the study of ragweed pollen. The technique yielded more distinct and reproducible patterns and allowed for simultaneous detection and identification of pigment, carbohydrate, peptide, and protein bands by visual observation and selective staining.

WSR resolved itself into eleven components in borate buffer, whereas MLL resolved itself into a lesser number of components. Regardless of the buffer used, MLL was always resolved into a smaller number of components than WSR.

Alcohol extraction, prior to water extraction, appeared to free the ragweed of much of its water-soluble pigments, which is in accordance with the findings of Abramson and associates.[44] The three faster migrating pigments of WSR were absent in MLL, but were present in ROH SOL ragweed.

The dialysate of WSR apparently contained all the components present in whole WSR, with the exception of the protein constituent. The dialyzed residue still contained a pigment which migrated with the diffuse protein band; it also stained slightly with ninhydrin. Thus, all the components identified in WSR can be accounted for in the dialysate plus the dialyzed residue. Since no new component was detected in the dialysate and dialyzed residue of WSR, it is highly unlikely that ragweed pollen constituents are constantly breaking down during dialysis, as suggested by Loveless and coworkers.[29]

There is a suggestion, however, of some alteration occurring in WSR on dialysis. The dialyzed residue stained for carbohydrate, although very weakly, in the area of the protein band, whereas the original WSR extract contained no carbohydrate-stainable material associated with the protein constituent. There are at least two explanations to account for these observations:

(1) Some configurational change might have taken place within the protein moiety of WSR during the extensive dialysis liberating carbohydrate stainable groups.

(2) The fact that carbohydrate-stainable material was detected in the dialyzed residue of WSR and not in the WSR itself may be due to a concentration effect. WSR may not contain a sufficient amount of the component necessary to be revealed by the carbohydrate reagent. On the other hand, this component might be concentrated to a relatively much higher level on dialysis.

The presence of detectable pigment migrating with the protein in the dialyzed residue and its apparent absence in the whole WSR could be explained in the same manner.

On the other hand, the total number of electrophoretically distinct components present in the dialysate and dialyzed residue of MLL exceeded the number found in undialyzed MLL. This finding suggests that degradation of larger into smaller molecules did occur in the MLL on dialysis. As in the case with the dialyzed residue of WSR, pigment was still present in the dialyzed residue of MLL which also migrated with the protein. These data imply that a pigment either is specifically bound to the protein or that it has similar electrophoretic properties.

The pH 6 SOL could not be resolved into more than one component by paper electrophoresis. One could interpret this finding in terms of a one-component system, the protein, carbohydrate, peptide, and pigment all being part of one and the same molecule which is resistant to alkaline hydrolysis.

On the basis of the sedimentation constants found for the various extracts, molecular weights conceivably could be calculated, provided that the diffusion

coefficient and partial specific volume were known for each fraction. However, diffusion coefficients and partial specific volumes are significant only when calculated for one-component systems. Since none of our fractions, except possibly the pH 6 SOL, complied with this requirement, molecular weight determinations were dispensed with. Due to the high concentration of NaCl in this latter extract and our inability to equilibrate it against the buffer by dialysis without loss of low molecular weight components, free electrophoresis on this material was not performed.

There is no doubt that a shift of small-molecular-weight material occurred through the Visking tubing on dialysis of WSR against water. This was reflected by the great discrepancy in the sedimentation constants of the dialysate and dialyzed residue of WSR. This latter finding served as evidence for the presence of some large molecular-weight material in ragweed pollen extract, which might have been masked by the relatively greater concentration of small molecular-weight material, when the whole extract was examined in the ultracentrifuge. This assumption seems reasonable in view of our experimental findings that 90 per cent by weight of the WSR dialyzed through Visking tubing.

The sedimentation constant of MLL was considerably greater than that of WSR, suggesting that some low molecular-weight material had been removed by the preliminary alcohol extraction. The low sedimentation constant of the ROH SOL supports this interpretation.

It is worth noting that ragweed pollen powder displayed cutaneous activity even after sixteen days of unabated extraction with water,* whereas the extracting fluid at the end of the sixteen days' extraction displayed no such activity. This observation suggested that allergenically active groups were still incorporated in the WSR INSOL residue. Since the WSR INSOL was active when in contact with tissue fluids during scratch test, the possibility of extracting this material with media similar in composition to tissue fluids was investigated. On incubating this material with normal human serum at 3 to 5° C., allergenically active material was detected in solution. Similar results were obtained on extraction of WSR INSOL with physiologic saline and phosphate buffers. Since, in the present study, we were mainly interested in the properties of WSR, the investigation of the properties of the WSR INSOL was discontinued for the time being.

As mentioned previously, the MLL INSOL ragweed, which exhibited no reactivity by scratch test, nevertheless was able to liberate active soluble material when extracted with 0.05 N NaOH for seventy-two hours. As it is highly inconceivable that sodium hydroxide would convert an inert material into an allergenically active one, a possible explanation for these findings would be that there exist residual active groups in the MLL INSOL fraction which are shielded in some way from direct contact with water and alcohol during extraction.

*As mentioned previously, the extracting fluid was replaced every two days.

In testing the various fractions for allergenicity on sensitive persons, scratch tests were performed. In spite of the fact that the intracutaneous test is far more sensitive, the scratch test was adopted in view of the greater danger of inducing constitutional reactions by the intracutaneous injection. For this same reason, no sensitive person was tested with more than seven to eight fractions at any one sitting. All tests were performed in duplicate. At no time were all the fractions tested on a single person at any one sitting. Since each sensitive person displayed his own pattern of reactivity to different fractions, it was impossible to evaluate on an absolute basis the activity displayed by each fraction.

To circumvent this difficulty, in subsequent studies on ragweed pollen extracts the passive transfer technique was used in preference to the scratch test.

SUMMARY

1. Defatted ragweed pollen was extracted with a number of solvents and the various fractions were analyzed by free and paper electrophoresis and ultracentrifugation. All fractions were compared for their allergenic reactivity by skin tests.

2. Extraction of defatted ragweed pollen with different solvents yielded fractions of different composition.

3. All the extracts soluble in the different solvents displayed allergenic reactivity, while the water-insoluble ragweed was the only one of the insoluble residues to display reactivity consistently.

4. The results of this study suggest that zone electrophoresis is suitable for the further fractionation of the various ragweed pollen fractions.

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STUDY ON PASSIVE CUTANEOUS ANAPHYLAXIS WITH SERUM OF ALLERGIC PATIENTS

J. Prochazka Fisher, M.D., Elliott Middleton, M.D., and
Arthur E. O. Menzel, Ph.D., New York, N. Y.

LOCAL passive cutaneous sensitivity of the guinea pig by transfer of human serum has been described by Ovary and Biozzi.[1] The reaction is called passive cutaneous anaphylaxis (PCA). It can be produced also with animal immune sera. Antiserum is injected intracutaneously and two to six hours later a mixture of antigen and a dye is injected into the circulation. A positive reaction is signified by the rapid accumulation of the dye in the subepidermal tissues surrounding the intracutaneous injection site. The antisera studied by Ovary and Biozzi were human precipitating and nonprecipitating diphtheria antitoxic antisera and agglutinating antityphoid and antibrucella convalescent sera. In the case of the antidiphtheria sera, it was shown that 0.13 mcg. antibody N (precipitating or nonprecipitating) was sufficient to sensitize guinea pig skin. The authors speculate that the PCA reaction "can also be used in all cases where one would use the Prausnitz-Küstner reaction," that is, the passive immediate wheal and erythema reaction in human skin, which is obtained by transfer of serum from induced as well as spontaneous human allergy. That this is not entirely correct is brought out in our study.

The purpose of the present experiments was to examine passive cutaneous anaphylaxis with the sera of patients with (1) the immediate wheal and erythema type of spontaneous hypersensitivity (hay fever), (2) serum sickness, (3) posttreatment ragweed-blocking antibody, and (4) rabbit antisera against alum-precipitated ragweed pollen extract. In addition, fractions of human ragweed-sensitive serum obtained by electrophoretic separation and starch block fractionation[2] were also tested.

Experimental.—Male albino guinea pigs, each weighing 300 to 400 grams, were used throughout the study.

Technique.—Shaved abdominal skin was employed for intracutaneous injections. Four to twelve intradermal sites were prepared, each with 0.1 ml. serum, and six hours later the intracardial injection of corresponding antigen-dye mixture (2 to 3 ml. total volume) was made. After twenty to thirty minutes the reactions were read and considered positive (+) if an accumulation of dye 1.0 cm. in diameter or greater appeared. In each test animal, a known

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positive reacting system and normal serum were included as controls. Each serum was examined for PCA at least twice. Serum dilutions were prepared with 0.85 per cent saline solution.

Antigens.—The sensitized animals were injected intracardially with proper antigen: ragweed extract, 70,000 to 140,000 protein nitrogen units (1 PNU = 0.01 mg. N); timothy extract, 50,000 PNU; horse epithelium extract, 50,000 PNU; undiluted horse serum, 0.4 ml.; and tetanus antitoxin (Lederle), 0.4 ml. Each antigen was mixed with 0.25 ml. per 100 grams of body weight of 0.5 per cent Evans blue solution just prior to injection.

Sera.—The various sera studied and the types of cases from which they were obtained are shown in Table I.

TABLE I. TABULATED RESULTS OF SERUM STUDIES

TYPE OF SENSITIVITY	ANTIGEN	NUMBER OF CASES	INTRACUTANEOUS IMMEDIATE REACTION		PRECIPITINS*	PASSIVE CUTANEOUS ANAPHYLAXIS
			DIRECT TEST	PASSIVE TRANSFER		
Spontaneous immediate	Ragweed	8	All positive	All positive	Negative	Negative
In man	Timothy	1	Positive	Positive	Negative	Negative
	Horse epithelium	4†	All positive	All positive	Negative	Negative
	Horse serum		All positive	All positive	Negative	Negative
Induced immediate	Tetanus antitoxin (Serum disease)	1				
In man	1/27/1955 Bleeding		Positive	Positive	Positive	Positive
	3/11/1955 Bleeding		Positive	Positive	Positive	Positive
	6/28/1955 Bleeding		Positive	Positive	Positive	Positive
In rabbits	Ragweed: Rabbit antiragweed serum	6	Positive (one done)	All positive‡	All positive	All positive
Induced blocking antibody In man	Ragweed	11	All negative	All negative	All negative	All negative

*Determined by agar gel diffusion (Oudin).
†Combined sensitivity to horse epithelium and horse serum.
‡Done in rabbits, not human beings.

In the spontaneous immediate wheal and erythema group, sera containing skin-sensitizing antibody gave positive Prausnitz-Küstner reactions in at least 1:800 dilution; those that showed combined sensitivity to horse epithelium and horse serum gave no history of prior horse serum administration.

The horse serum sickness patient had suffered serum sickness following administration of horse tetanus antitoxin. Of the many bleedings three were examined in PCA experiments (Jan. 27, 1955, March 16, 1955, and June 28, 1955). Dilutions of January 27 serum were prepared before and after heating at 56° C. for various periods of time and then were tested for precipitins by Oudin agar gel diffusion,[3] Prausnitz-Küstner reaction, and passive cutaneous anaphylaxis in guinea pig skin.

Six rabbit antisera containing precipitins against ragweed were included in the induced sensitization group.

In the posttreatment ragweed sera used, containing blocking antibody,[4a] the blocking activity was found to be as high as 200 to 500 PNU per milliliter. Three of these sera were obtained from nonallergic persons given ragweed extract injections; the skin-sensitizing activity of the remaining eight sera was destroyed by heating at 56° C. for four hours.

The fractions of ragweed-sensitive sera consisted of albumin, total globulin, gamma globulin, pigmented beta globulin, and "slow beta globulin."[6]

RESULTS

In Table I are shown the results for all sera of direct intracutaneous testing of the patient, the Prausnitz-Küstner reactions, precipitins, and PCA reaction. It can be seen that none of the sera of the *spontaneous* immediate wheel and erythema sensitivity, including pollens, horse serum, and horse epithelium, were capable of producing sensitization of guinea pig skin. Positive PCA reactions were obtained only with sera from the *induced* sensitization group. All sera in this group were positive. The human sera with *induced blocking antibody* to ragweed did not produce positive PCA reactions, however, and precipitins were not found in these sera, although each had proved to have good blocking activity by inhibition tests.[7]

TABLE II. EFFECT ON PRECIPITINS, PRAUSNITZ-KÜSTNER REACTION AND PASSIVE CUTANEOUS ANAPHYLAXIS USING HORSE SERUM SICKNESS SERUM HEATED AT 56° C. FOR VARIOUS TIMES

DURATION OF HEATING AT 56° C. (HOURS)	PRECIPITINS*	PASSIVE TRANSFER IN HUMAN SKIN DILUTIONS			PASSIVE CUTANEOUS ANAPHYLAXIS IN THE SKIN OF GUINEA PIGS (DILUTIONS)								
		NONE	1-10	1-100	1-10	1-100	1-200	1-400	1-800	1-1,600	1-2,000	1-3,200	1-6,400
Unheated	+	+	+	±	+	+	+	+	+	+	+	+	+
4	+	+	-	-	+	+	+	+	+	+	+	+	+
8	+	±	-	-	+	+	+	+	+	±†	-	-	-
12	+				+	+	+	+	-	-	-	-	-
28	+				+	+	±†	-	-	-	-	-	-
48	+				+	±†	-	-	-	-	-	-	-
60	+				±	-	-	-	-	-	-	-	-

*Determined by agar gel diffusion (Oudin).
† ±reaction represents an accumulation of dye smaller than 1 cm.

Negative results were obtained with all the serum fractions of ragweed-sensitive patients.

The effect on precipitins, Prausnitz-Küstner reaction, and PCA of heating the January 27 serum of the horse serum sickness case is shown in Table II. The data show that precipitins measurable by the Oudin method withstand heating at 56° C. for sixty hours. A marked decrease in precipitin content was evident after four hours' heating, as demonstrated clearly by classical test tube precipitation. The Prausnitz-Küstner reaction in human skin became negative after eight hours' heating but the PCA reaction remained positive at 1:10 dilution after sixty hours' heating.

*Injection of pollen extracts into normal persons or hay fever subjects stimulates the development of immune or "blocking" antibodies.[4] The word "blocking" in this connection applies to the behavior of these antibodies in skin tests and must not be confused with the use of the term "blocking" in the serologic sense.[5]

DISCUSSION

From the data of these experiments, it is apparent that sera from spontaneously allergic patients with immediate wheal and erythema type of sensitivity to pollen antigens, horse epithelium, and horse serum are not capable of sensitizing guinea pig skin if the technique of PCA as used in the present study is employed. The ragweed-sensitive serum fractions obtained by zone electrophoresis also did not display and passively transferred reactivity not brought out by whole sera. These findings are not in complete accord, therefore, with claims that the PCA is identical with the Prausnitz-Küstner reaction[1] and that it could be obtained with antisera containing spontaneous human skin-sensitizing antibodies (reagins).[8] Both PCA in guinea pigs (an anaphylactic reaction) and the Prausnitz-Küstner transfer in men (a reaginic or an anaphylactic reaction) are due to passively fixed antibody. In both passive transfers the local cellular sensitivity follows fixation of anaphylactic antibody, whereas the fixation of reagins is peculiar to the Prausnitz-Küstner reaction in human skin.

In cases of combined spontaneous horse epithelium and horse serum sensitivity and in the induced horse serum sickness case, each exhibited immediate direct intracutaneous reactivity and each gave a positive Prausnitz-Küstner reaction to horse serum, whereas only the precipitating serum-disease serum gave a positive PCA reaction. Hence, it seems that the PCA reaction is further evidence for the distinction between the *spontaneous* immediate wheal and erythema type of sensitivity (antibodies characterized by heat lability and human skin-sensitizing property) and the *induced* immediate wheal and erythema type of sensitivity (anaphylaxis) characterized by antibodies that have heat stability, human skin-sensitizing property, and smooth muscle-sensitizing ability. The latter is demonstrable in passive systemic anaphylaxis and Schultz-Dale phenomena in guinea pigs.

In the present experiments, positive reactions were obtained only with human or rabbit antisera which contained precipitins. However, the PCA reaction cannot be ascribed entirely to the presence of a precipitating antibody. It has been reported that human nonprecipitating diphtheria antitoxin can transmit sensitivity to guinea pig skin as well as precipitating antitoxin.[1] On the other hand, the serum-sickness serum heated eight hours at 56° C. lost its ability to sensitize human skin but retained its anaphylactic property and still contained precipitins. There is evidence, furthermore, that gamma globulin of different species has different binding affinities for guinea pig skin as demonstrated by reversed PCA experiments[9]; thus, rabbit and human gamma globulin are bound to guinea pig skin, whereas horse,[1] bovine, and goat gamma globulin are not. The aforementioned observations offer two possible explanations for the failure of human ragweed-blocking antibody to give the PCA reactions: (1) either ragweed-blocking antibody is a human gamma globulin[7, 10] not capable of sensitizing guinea pig skin (as do diphtheria anti-

toxin, antityphoid, antibrucella, and antihorse serum gamma globulin) or (2) the lack of this sensitizing activity constitutes evidence for its immunologic differences from anaphylactic antibodies.

SUMMARY

Antibodies of spontaneous (reaginic) and induced (anaphylactic) immediate wheal and erythema type of sensitivity and human ragweed-blocking antibody were tested for their ability to induce passive cutaneous anaphylaxis (PCA) in guinea pig skin.

1. None of the sera of the *spontaneous* immediate wheal and erythema sensitivity (against pollens, horse epithelium, and horse serum) were capable of producing passive sensitization of guinea pig skin, although each exhibited a strong positive Prausnitz-Küstner reaction.

2. Positive PCA was obtained only with sera from the *induced* type of immediate wheal and erythema sensitivity against horse serum in human beings and ragweed pollen in rabbits. However, the human sera containing *induced blocking antibody* to ragweed did not produce positive PCA reactions, although each manifested good blocking activity in inhibition tests.

Thus, the PCA in the guinea pig by transfer of human serum could be considered as evidence in favor of immunologic distinction between antibodies of the *spontaneous* immediate wheal and erythema type of sensitivity (human skin antibodies known as reagins), antibodies of the *induced* immediate wheal and erythema type of sensitivity (skin-sensitizing antibodies with anaphylactic property), and the human *blocking antibodies.*

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STOMATITIS FROM ALLERGY TO ACRYLIC DENTURE

Report of a Case

Louis Tuft, M.D., and G. Frank Santor, M.D., Philadelphia, Pa.

IRRITATION or inflammation of the oral mucosa resulting from the use of plastic dentures has occurred with increasing frequency since the introduction of the acrylic plastic materials as a substitute for vulcan or rubber in the preparation of artificial dentures. The irritation sometimes is due to improper fitting of the denture, which usually can be corrected by the dentist. When it persists, the irritation may be due to the presence in the denture of a small amount of the liquid monomer of acrylic resin, which is irritating and sensitizing.

Artificial plastic dentures usually are prepared by mixing the liquid monomer of acrylic resin, called methyl methacrylate, with the powdered polymer, designated polymethyl methacrylate. The mixture then is heated until further polymerization occurs. After subsequent hardening, the familiar pink-colored denture is formed. If the denture is properly heated, or "cured" as the dentist terms it, complete polymerization takes place and none of the irritating monomer is left behind. If it is improperly "cured," however, the presence of the monomer, even in small amounts, may cause irritation and/or sensitization.

Allergists seldom see patients who have irritation or sensitization of the oral mucosa. When they do, the diagnosis of specific allergy is not easy and may be suspected only because the stomatitis disappears when the patient does not wear the denture for awhile and recurs shortly after the reinsertion of the plate. Recently we saw a patient with stomatitis in whom we were able to obtain definite proof of a specific allergy. Because this condition is so uncommon, we thought it might be worth reporting.

CASE REPORT

Mrs. H. F., aged 63 years, first came to the Medical Outpatient Clinic of Temple University Hospital on April 22, 1955, complaining of swollen gums and burning and tingling sensations of the tongue following the use of an upper artificial denture. She was in excellent health until September, 1954. At that time, and shortly after her upper teeth had been extracted, she began to wear an artificial denture made of plastic material. Symptoms were noted within a short time after inserting the denture and disappeared twenty-four to forty-eight hours after removing it. She returned to her dentist, who attributed the difficulty to mechanical difficulty with the denture. Repeated adjustments and attempts to alter the denture failed to improve the symptoms. In November, 1955, she

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went to another dentist in Allentown, who gave her a second denture made of similar plastic material which supposedly was properly "cured." Repeated attempts to use the new denture were followed by exactly the same symptoms, which came on within a half-hour after inserting it. The patient stated that if she wore the denture for a longer period of time, she developed the usual oral symptoms but, in addition, noted nausea and itching of the skin with a few visible hives.

Aside from these manifestations, her general health had been good. She had occasional attacks of dizziness when constipated, but not if her bowels were kept regular. Prior to the removal of her teeth, she had slight pains in the proximal phalanges and in the wrist joints, which were diagnosed as rheumatoid arthritis. These symptoms disappeared following the removal of her teeth in August, 1954. She had pneumonia at the age of 18, with no sequelae, and several operations, including the removal of both tubes and ovaries, appendix, uterus, and tonsils. She is intolerant of certain drugs, particularly those in the

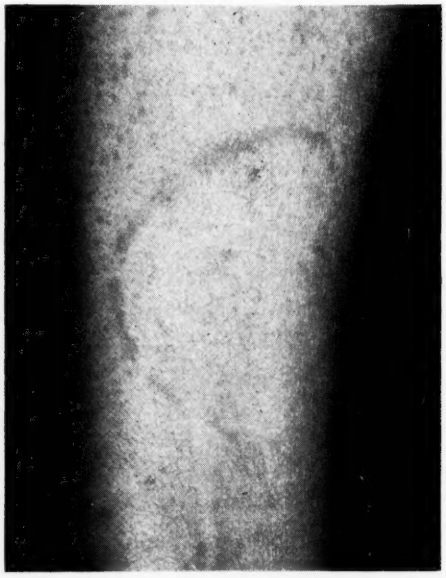

Fig. 1.

opium group. The ingestion of morphine on one occasion produced immediate marked constriction of the chest and throat and an inspiratory croup. The ingestion of clams caused nausea and vomiting. A grandson is allergic to milk. Three sisters and one brother are allergic to various drugs. On one occasion, her mother had marked acute anaphylactic shock after morphine. The patient worked in an office, smoked occasionally, and used feather and foam rubber pillows. The use of plastic pillowcases seemed to induce sneezing and dryness in the nose so that she had to discontinue their use.

Physical Examination.—Blood pressure was 140 systolic and 86 diastolic. There was mild obesity and evidence of bilateral corneal scars. The membranes of the nose were pale but not boggy. Tongue and pharynx were negative. The mucous membranes of the upper alveoli were slightly erythematous and showed several pin-point vesicles. Examination of the lungs revealed no abnormalities. The heart seemed slightly enlarged to the left with an audible soft systolic nontransmissible mitral murmur. The abdomen was soft, and showed scars of previous operations. There were no abnormalities of the skin, and the

reflexes were normal. Routine laboratory examinations were negative. A diagnosis of probable allergic stomatitis from an acrylic denture was made, and the patient was referred to the allergy clinic for further study.

The patient reported to the allergy clinic on May 2, 1955. At that time she had no oral symptoms, since she had not worn the denture for several days. However, application of the edentulous portion of the plate to her arm for a period of eight hours produced a red line outlining the outer rim of the denture that had been in contact with the skin (Fig. 1).

Since the denture was made of processed acrylic resin, we obtained some of the liquid monomer (methyl methacrylate) from the Temple School of Dentistry for patch tests. In addition, we tried to scrape some material directly from the denture to use in the test, but little actually was obtained because we were afraid of altering its contour and fit. Patch tests were done on the arm with both these materials, and the patches were allowed to remain for forty-eight hours. A positive patch test was obtained only with the methyl

Fig. 2.

Fig. 3.

methacrylate, but not with the scrapings (Fig. 2). On May 9 another direct pressure test with the plate was done, the patient being instructed to leave it on more firmly and for a longer period of time. After being in contact with the skin for about twelve hours, the denture was removed. A positive reaction, consisting of red lines duplicating the points of contact, was noted (Fig. 3). In addition to the local reaction, both after the patch test with the methacrylate and the direct pressure test with the denture, the patient reported the development of soreness of the roof of the mouth and tongue which lasted for a short time. Following the demonstration of the unsuitability of plastic dentures because of her allergy to the acrylic material, the patient was referred back to the dental school, where a vulcan denture was made for her. After a short period of readjustment, the patient has worn this new denture apparently without difficulty, at least until last heard from in mid-September, 1955.

COMMENT

The fact that the artificial plastic denture was responsible for the irritation of the oral mucosa was established in this patient by the clinical history

of symptoms of stomatitis after repeated attempts to wear it, followed by relief when the denture was removed. Repeated adjustments of the first denture and even the preparation of a second denture failed to prevent the irritation, suggesting that it was not due to either improper fitting or "curing" but perhaps to a true allergy. Suspicion of this was obtained by the initial positive reaction to the pressure test done with the denture, but more definite proof was furnished by the positive patch test to the liquid monomer.

In an excellent review published in September, 1954, Fisher[1] discussed the question of sensitization of the skin and oral mucus membranes to acrylic denture materials. He reported four patients with allergic contact dermatitis of the skin and hands, proved clinically and by patch tests to be due to allergy to the liquid monomer of acrylic acid. Included in this report was the study of twenty cases (all women) of sore mouth caused by acrylic dentures. Four of these patients were told that they were allergic to the dentures because of positive reactions obtained after strapping the plates on the forearm for forty-eight hours. But only one of the four had a specific sensitization as proved by a positive patch test to the liquid monomer and even she could wear a properly heat-cured denture without difficulty. Seventy control patients, including twenty men and fifty women, had plastic dentures strapped to their forearms for forty-eight hours; all reacted with various degrees of redness and papulation. In two men and fifteen women, numerous bullae formed at the points of maximum pressure. The histologic examination of these bullae showed no eczematous responses. By contrast, of 100 control patients who were patch tested with the liquid monomer (methyl methacrylate), only one showed a slight redness after forty-eight hours, indicating that the monomer itself was not irritating. Fisher concludes, from these studies, that true allergic sensitization to the acrylic denture material is extremely uncommon; also that it is not advisable to depend upon tests done with the entire denture for determining such sensitization, since many persons react to it in a nonspecific way. The best method for testing is by patch test with the methyl methacrylate liquid monomer. When the latter is completely polymerized, it is no longer a sensitizer or eliciter of allergic reactions. No instance of allergic sensitization to the heat-cured acrylic denture material was found in twenty patients with sore mouths caused by dentures. Self-cured acrylic denture material, however, sometimes contains sufficient unpolymerized monomer to give reactions in patients sensitized to the monomer.

The patient which we are reporting had a positive pressure test with the denture. This, according to Fisher, is not in itself diagnostic and may be nonspecific. But she also had a positive patch test to the monomer, which is indicative of the existence of true allergic sensitization rather than of nonspecific irritation. This was substantiated clinically by the fact that the patient could wear a vulcan denture without further difficulty.

One question which suggests itself concerns the manner in which the sensitization occurred in this patient. Allergic sensitization to chemical agents like acrylic resin usually is acquired by contact. While the history is uncertain, evidence of the sensitization in our patient followed shortly after she began

wearing the denture so that the period of contact must have been quite short. The presence of other manifestations of allergy in this patient perhaps might have enhanced her tendency to sensitization; thus, she had a severe allergic reaction to morphine, as did her mother; furthermore, she could not tolerate a plastic pillowcase without sneezing. It is possible that she possessed a natural allergy to plastics, but that is difficult to prove, as is also the relationship between her allergy to drugs like morphine and the allergy to the acrylic resin.

SUMMARY

The present report is of a patient with allergic stomatitis resulting from sensitization to acrylic denture materials. The presence of specific allergy in this patient, suspected from the clinical history and from a positive direct pressure test with the denture on the arm, was definitely proved by means of a positive patch test to the liquid monomer of the acrylic resin (methyl methacrylate) used in the preparation of the plastic denture, and clinically by the relief afforded by wearing a vulcan denture. The necessity for reliance upon a positive patch test with the liquid monomer rather than upon clinical evidence or pressure tests with the denture in establishing the diagnosis of allergy is emphasized.

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Editorial

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

SUFFERERS from allergic diseases and their physicians will welcome the news that the United States Public Health Service has officially recognized the need for more extensive investigation of the nature and treatment of these common and troublesome illnesses. In the fifty years since the concept of allergy was proposed by von Pirquet, much has been learned, but it is apparent that far more knowledge is needed. Much of the early research in this field was carried out by physicians actively engaged in practice, and often hampered by lack of time and training in the basic sciences. Few full-time investigators have devoted their attention to allergy.

With the rapid advances in the techniques of medical investigation, the amateur research in allergy has failed to keep pace with the progress being made in other fields of medicine. Full application of the many new methods of immunochemistry, enzyme chemistry, tracer studies, and pulmonary physiology requires both elaborate apparatus and highly specialized investigators. This was recognized by the national allergy societies when they joined forces to launch the American Foundation for Allergic Diseases.

The timely decision of the Public Health Service to include allergy in its highly successful program of medical investigation promises to advance greatly the type of work done in this field. The old National Microbiologic Institute at Bethesda, Maryland, has been renamed the National Institute of Allergy and Infectious Diseases by Surgeon General Leonard A. Schiele, and it has been assigned the additional duties of research in allergy.

"The renaming of the Institute," Dr. Schiele said, "reflects the importance of the new research program on allergies and the close relationship of such research with the study of infectious diseases."

The Institute will conduct an intramural program at Bethesda and also grant aid to research projects in allergy being conducted by hospitals and medical schools in other parts of the country. A separate national advisory council on allergy and infectious diseases is being set up to make recommendations to the Surgeon General regarding the Institute's new activities.

American Academy of Allergy

HISTORIAN'S REPORT

THE twelfth annual meeting of the American Academy of Allergy was held at the Chase Hotel, St. Louis, Missouri, Feb. 6, 7, and 8, 1956. The total registration was 446, with 150 fellows, 132 members, and 164 guests attending. There were 124 exhibit personnel staffing forty-three commercial exhibits. In addition, there were eight scientific exhibits.

A preconvention scientific assembly was held at the Chase Hotel on February 4 and 5. This was sponsored by the Subcommittee on Postgraduate Education under the chairmanship of Dr. Charles H. Eyermann.

On February 5 a meeting of the Association of Allergists for Mycological Investigations was held.

The scientific program consisted of twenty-five papers representing original investigative work in the various fields of clinical allergy. There was a panel discussion on the recent developments in the treatment of allergic diseases with steroid hormones. By means of a grant by Ciba Pharmaceutical Products, Inc., the scientific meeting was supplemented by a series of addresses: "The Gamma Globulins" by Charles A. Janeway, M.D., of Harvard University Medical School; "Some Mechanisms Involved in Allergic Contact Dermatitis" by Herman N. Eisen, M.D., of the Washington University School of Medicine; "Immunohematology" by Carl V. Moore, M.D., of the Washington University School of Medicine; "Bacterial Hypersensitivity" by Sidney Raffel, M.D., of the Stanford University Medical School; and "Foods, Drugs, Cosmetics and the Allergist" by Irvin Kerlan, M.D., Associate Medical Director, Food and Drug Administration, Washington, D. C. There was an address by Lawrence J. Halpin, M.D., President of the American College of Allergists, entitled "An Answer to a Question."

The President's Luncheon was held on Tuesday, February 7, and at this occasion the President, Dr. Stanley Hampton, announced the formation of the National Institute of Allergy and Infectious Diseases as one of the National Institutes of Health, United States Public Health Service, Department of Health, Education, and Welfare.

At the International Luncheon five countries, in addition to the United States, were represented.

At the Business Meeting, held in the afternoon of February 7, the executive secretary, James O. Kelley, reviewed the growth of the Academy since 1948, when the total membership was 528. The total membership of the Academy is

now over 1,000. Twenty-six members were advanced to fellowship and sixty-seven new members were elected. Announcement was made of the Post-Convention Hawiian Tour following the 1957 meeting in Los Angeles.

The Hostess Committee was under the chairmanship of Mrs. Joseph W. Noah, and the Academy is most grateful for their contribution. On the evening of February 6 the Schering Corporation held their annual cocktail party on the Starlight Roof of the Chase Hotel, and on the evening of February 7 the Academy was entertained at Anheuser-Busch, Inc. Also, many members enjoyed the splendid hospitality offered in the homes of several St. Louis allergists.

The thirteenth annual meeting will be held in the Statler Hotel, Los Angeles, California, Feb. 4, 5, and 6, 1957.

HOMER A. HOWES, Historian.

NEW MEMBERS AND FELLOWS

At the twelfth annual meeting of the American Academy of Allergy in St. Louis, Missouri, Feb. 6, 7, and 8, 1956, the following physicians were elected to membership in the Academy:

Jack Barrow
110 South Central Ave.
Clayton 5, Missouri

Louis Beresh
Veterans Administration
310 East Jefferson Ave.
Detroit 31, Michigan

Irwin L. Bernstein
Northwestern University Medical School
303 East Chicago Ave.
Chicago 11, Illinois

Jerome Bernstein
881 Lafayette St.
Bridgeport, Connecticut

George H. Berryman
1421 Maple Ave.
Evanston, Illinois

Abraham Bolker
2885 SW Third Ave.
Miami 45, Florida

Marcio M. Bueno
132 School St.
New Bedford, Massachusetts

Irvin Caplin
3120 North Meridian St.
Indianapolis 8, Indiana

Catherine C. Carroll
5365 West Devon Ave.
Chicago 30, Illinois

Emanuel Corwin
5 Richards Ave.
Dover, New Jersey

George Dean
425 East Wisconsin Ave.
Milwaukee, Wisconsin

R. Dale Dickson
403 National Reserve Bldg.
Topeka, Kansas

A. J. Edelstein
408 Lincoln St.
Johnstown, Pennsylvania

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Edward J. Wallick
Long Beach Veterans Administration Hospital
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Foreign Members

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Yrjo Abraham Hongisto
Porthanink 5B
Helsinki, Finland

Miguel Miranda-Long
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Armando J. Silicani
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The following applicants were advanced to fellowship:

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Toledo 2, Ohio

George S. Bozalis
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H. Elias Diamond
1749 Grand Concourse
Bronx 53, New York

Oscar Feinsilver
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Worcester 8, Massachusetts

Lois Frayser
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Seattle 1, Washington

William C. Grater
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Dallas, Texas

Lowell Henderson
Mayo Clinic
Rochester, Minnesota

William A. Howard
1517 30th St.
Washington 7, D. C.

Macy I. Levine
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Pittsburgh 13, Pennsylvania

Ben Miller
1433 Gregg St.
Columbia 1, South Carolina

Harry L. Mueller
31 Church St.
Winchester, Massachusetts

James C. Overall
1924 Church St.
Nashville, Tennessee

Carroll M. Pounders
1220 North Walker St.
Oklahoma City 3, Oklahoma

Harold Rand
515 N.E. Fifteenth St.
Miami 32, Florida

Charles E. Reed
530 North 27th St.
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Roland B. Scott
1114 Girard St., N.W.
Washington, D. C.

Jerome Sherman
2502 Eutaw Pl.
Baltimore 17, Maryland

Sheldon C. Siegel
5830 Overhill Dr.
Los Angeles 43, California

Arthur H. Sussman
345 Bloor St., West
Toronto, Ontario

Richard D. Wiseman
608 East Genesee St.
Syracuse 2, New York

Charles P. Wofford
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Johnson City, Tennessee

Foreign Fellowship

Clarence Y. Sugihara
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Honolulu 14, Hawaii

The following applicants were elected to affiliate fellowship:

Helge Colldahl
Medical Clinic II
St. Goran's Hospital
Stockholm K. Sweden

Paul Ward Farmer
98 Collins St.
Melbourne, Australia

Ake Nilzen
Jarlaplan 4
Stockholm, Sweden

REPORT OF THE RESEARCH COUNCIL

THE year 1955-56 has been a year of satisfactory growth of the Research Council. Its active committees have clarified the purpose and scope of their projects and stepped up their activities in promising areas. The committees and subcommittees which have been inactive have re-examined their difficulties and offered constructive suggestions for reactivation or have functioned in an advisory capacity.

I. COMMITTEE ON AIR-BORNE ALLERGENS

The Committee on Air-borne Allergens consists, at present, of four subcommittees: (1) the Subcommittee on Pollen and Molds, (2) the Subcommittee on Dust, (3) the Subcommittee on Insects, and (4) the Subcommittee on Weather.

1. *Subcommittee on Pollen and Molds*

Herbarium.—Our type collection of pollens, cared for by Dr. Ralph F. Voigt and his assistant, Floyd A. Swink, has furnished study material for workers within and without the Academy as in past seasons. Additions of plant and pollen specimens during the past year have come only from our staff. It should be noted again that the University of Illinois cares for the herbarium and sends out slides and sometimes pressed plants without any charge to the Academy of Allergy or to those who request service.

Statistical Report.—The 1954 and 1955 statistical pollen reports were sent out to all members and collaborators and to all physicians who have personally requested copies. About 8,000 copies of the 1954 revision of the Ragweed Pollen Index (Hay Fever Holiday) were distributed to medical schools, physicians, automobile clubs, and laymen during the summer and fall of 1955. Articles and notices concerning this pollen index of more than 600 places in North America appeared in a number of publications, including the *Consumer's Report* and *McCall's Magazine*. *Science Digest* reprinted, in the March issue, the secretary's article, "What the Pollen Count Means to You," from *Today's Health* (August, 1954).

Exhibits.—Pollen exhibits, including promotion of pollen and mold surveys, were given by the secretary, sometimes assisted by other members of the subcommittee, at the following meetings and museums:

American Academy of Allergy, New York, New York
American Medical Association, Atlantic City, New Jersey
Chicago Medical Society, Chicago, Illinois

American Medical Association (Clinical Session), Boston, Massachusetts

Interstate Postgraduate Medical Association, Milwaukee, Wisconsin

Museum of Science and Industry, Chicago, Illinois (Continuous from June to October)

Cleveland Health Museum, Cleveland, Ohio (Continuous from June to October)

Plans for 1956.—The two co-secretaries of the subcommittee, Mr. Durham and Dr. Merksamer, are invited to and plan to give an exhibit on molds at the Chicago Meeting of the American Medical Association in June, 1956.

Weed Control.—The problem of keeping ragweed from spreading in western Oregon is still a live issue. We were recently called on by the City Club of Portland for advice and educational material.

The Research Council recognized the increasing importance of ragweed control and asked Mr. Durham to prepare a summary of the scope and limitations of the problem. Mr. Durham sent the following outline which, by unanimous vote of the Research Council, is incorporated in this report.

THE STATUS OF RAGWEED CONTROL

To destroy all live ragweed in a limited area of tilled land is a comparatively easy assignment, but to prevent the appearance of more ragweed in the same area during subsequent seasons is often very difficult. Soil, once contaminated with ragweed seeds, may continue to sprout new plants from old seed for more than thirty years. Moreover, complete destruction of ragweed in a given local area does not prevent ragweed pollen contamination of the air above that weed-free area. Thus, ragweed pollen poses a public health problem in three dimensions.

Throughout agricultural states of the Mississippi Basin thousands of tons of pollen are picked up by ascending thermal currents and carried to elevations of 4,000 feet or more. With lateral wind currents averaging ten miles per hour, and with accelerations up to thirty-five miles per hour or more, vast quantities are known to shift for long distances into weedless, wooded areas. The buoyancy of ragweed pollen may be appreciated by noting that its rate of settling in still, dry air is only 100 feet per hour. Even Lake Michigan, with its sixty-mile-wide expanse of water, does not constitute a barrier to the eastward transport of toxic quantities of ragweed pollen by westerly winds. This has been proved by repeated air sampling at various points and at various altitudes over the lake.

Our present practical standards of clean crop farming in the Mississippi Valley and the high cost of more intensive spraying prevent effective reduction of pollen contamination of the air by weed control methods, except very locally at ground level. Neither does crop rotation in this area solve the problem.

The wide, heavily wooded Appalachian region would, as a distance barrier, partially protect the Atlantic Seaboard states from the pollens of the Mississippi-Ohio Basin. But the interspersed tilled areas of the Appalachians and the broad farmlands of the coastal region are sufficient to maintain active air contamination in all of the eastern states. Ragweed control is feasible only in the heavily forested regions of upper New York State, upper New England, and

Eastern Canada. Here, in localities where only 5 per cent or less of the land is under cultivation, intensive local control efforts should be tried, but always with atmospheric tests used as a control procedure in assessing results.

Ragweeds do not thrive in any area with less than twenty inches of annual rainfall. Thus, the intermountain states from the Rocky Mountains to the Sierras and Cascades have no appreciable ragweed pollen problem except where irrigation encourages the growth of ragweeds. Accidental introduction of the weeds into irrigated areas previously ragweed-free is a constant hazard, but chemical spraying and crop rotation should be successful, if started soon enough.

Abundant winter and spring rainfall on the north Pacific coast favors the growth of grass and other natural vegetation which quickly chokes out ragweed, even when introduced. In such areas local ragweed control is successful if crop rotation and spraying are followed faithfully. Ragweeds cannot compete with the semitropical vegetation of southern Florida.

Allergists are in favor of ragweed control. There are several serious counts against ragweeds besides their role in allergy. Indeed, allergists are ready to encourage any practicable public health measures that may be developed for an all-out fight against the ragweed. Yet, their long and constant experience with clinical hay fever and their extensive researches in pollen dispersal make them extremely conservative about any appreciable reduction of air-borne pollen in the heavily infested areas of this country by means of the herbicides now available.

Sunland and Tujunga.—Some time during 1953, an article appeared in *Reader's Digest* giving high praise to the villages of Sunland and Tujunga, California, adjacent to Los Angeles, as almost perfect resorts for those afflicted with hay fever and asthma. The writer of the article claimed that extensive atmospheric tests had proved the absence of pollen in the air, and even of smog, in the mountain area where these villages are located. Frequent inquiries have come to us regarding the validity of these claims. A field examination by the secretary on Oct. 21, 1955, revealed heavy growths of Russian thistle, false ragweed, Bermuda grass, and several other wind-pollinated plants in both villages. Smog seemed as heavy that day as in Los Angeles or Long Beach. Our plan is to institute pollen counting this season in one or both villages.

Sugar Beet Pollen.—A field survey was carried out by the secretary during the fall (1955) in the St. George area of southwestern Utah in conjunction with atmospheric studies on sugar beet pollen in that locality by Dr. George A. Peck of Salt Lake City. Dr. Peck will submit a complete report on this project at a later date.

Officers, Members, and Collaborators.—Oren C. Durham (Secretary for Pollen), David Merksamer (Secretary for Molds), Clarence Bernstein, Jose L. Cortes, A. O. Dahl (Technical Consultant), Sidney Friedlaender, Frank T. Joyce, Eloise W. Kailin, Dean A. Moffat, Frank Perlman, Roland T. Rooks, Bram Rose, Grace M. Talbott, Abraham M. Targow, Albert M. Tocker, Lester C. Todd, Martyn A. Vickers, Ralph F. Voigt (Curator of the Herbarium), Charles H. A. Walton, and Matthew Walzer.

About 100 allergists, public health officers, and botanists are carrying out local surveys throughout North America. The volume of figures for 1955 has become larger than for any previous year. Mold counts are being received from 32 stations.

2. *Subcommittee on Dust*

Activities.—In 1956, the Dust Committee acted as a clearinghouse for individual research on dust. Its activities consist of: (1) a continuation of efforts to separate out immunologically reactive components of house dust extract, using the agar diffusion technique; (2) immunologic characterization of dust extracts from different sources; (3) a continuation of efforts to find among bacteria substances similar to or identical with immunologically reactive components of house dust extract; (4) efforts to determine the conditions under which freshly ginned cotton acquires allergic and immunologic activity similar to that found in house dust extracts.

It might be of interest to members of the Academy that in the course of the studies, it became apparent that clear-cut differences exist between the allergic reactivity of house dust obtained from Endo Products, Inc., and Hollister-Stier Laboratories.

Officers and Members.—Francis C. Lowell (Secretary), Irving W. Schiller, and Max Samter.

3. *Subcommittee on Insects*

Activities.—Dr. Frank Perlman's Subcommittee on Insects is still in its formative stage. Dr. Perlman furnished a number of extracts of insects to interested allergists. He felt, however, that the activation of a committee should be deferred; but suggested that an informal meeting be called for the purpose of exchanging ideas and plans about the development of this field.

4. *Subcommittee on Weather*

Activities.—The activities of the Subcommittee on Weather have met with an immediate and sustained response of membership. The project is an attempt to study the effect of climatic change on the course of allergic diseases, particularly bronchial asthma. In cooperation with the United States Weather Bureau, individual observation posts (to date, 31) have been established throughout the United States at locations where Federal climatologists are available for consultation and guidance. The subcommittee acknowledges gratefully the cooperation of Mr. H. E. Landsberg, Chief of the Climatological Services Division of the Weather Bureau, United States Department of Commerce, Washington, D. C., and particularly of Dr. Gerald L. Barger, Central Area Climatologist, Agronomy Building, Iowa State College, Ames, Iowa, who has assisted us in the design and in the organization of the study. Contact between the climatologists and allergists who have expressed their willingness to cooperate in the project has been established. The most urgent problem of the subcommittee is the design of a form for a day-by-day (or perhaps one should say hour-by-hour) recording of the clinical variations in bronchial

asthma. The form should be sufficiently accurate to permit transfer on IBM cards (similar to or identical with those used by the Weather Bureau for the recording of climatological data) and statistical analysis.

Officers, Members, and Collaborators.—Harold S. Tuft, Medical Director of Jewish National Home for Asthmatic Children, 3447 West 19th St., Denver 4, Colorado, Secretary; Johnny A. Blue, Oklahoma City, Oklahoma; M. H. Brodkey, Omaha, Nebraska; Alan G. Cazort, Little Rock, Arkansas; Armand E. Cohen, Louisville, Kentucky; Victor L. Cohen, Buffalo, New York; Stanley Cohen, New Orleans, Louisiana; Seymour B. Crepea, Madison, Wisconsin; Murray Dworetzky, New York, New York; William S. Eisenstadt, Minneapolis, Minnesota; H. M. Farmer, Burlington, Vermont; Alan R. Feinberg, Chicago, Illinois; John K. Fulton, Wichita, Kansas; Frank F. Furstenberg, Baltimore, Maryland; Morton L. Hammond, Miami, Florida; M. Coleman Harris, San Francisco, California; John L. Jacobs, Atlanta, Georgia; Eloise W. Kailin, Washington, D. C.; Bennett Kraft, Indianapolis, Indiana; Macy I. Levine, Pittsburgh, Pennsylvania; John H. Mitchell, Columbus, Ohio; Dean A. Moffat, Salt Lake City, Utah; Joseph W. Noah, St. Louis, Missouri; Frank Perlman, Portland, Oregon; David McK. Pipes, Asheville, North Carolina; Maurice S. Segal, Boston, Massachusetts; Edward E. Seidmon, Plainfield, New Jersey; John M. Sheldon, Ann Arbor, Michigan; S. B. Steen, Tucson, Arizona; J. E. Stroh, Seattle, Washington; Joel D. Teigland, Des Moines, Iowa; J. Warrick Thomas, Richmond, Virginia.

5. *Subcommittee on Air Filtration*

Activities.—The Air Processing Committee has continued its efforts to design, arrange for, and supervise tests of the efficiency of various air filtration units. One of the Eastern manufacturers submitted a room air conditioner for evaluation; tests were carried out by the Farr Company and established that the filter was inadequate.

A growing volume of correspondence about specific problems, for example, the dust control of bedrooms in homes with forced air or gravity air heating systems, might make it feasible to expand the subcommittee and add a number of regional consultants to its central testing area. It was also suggested that a series of guides to the proper selection of filters and air conditioners be prepared which might be distributed to the membership.

Officers, Members, and Collaborators.—Dr. Willard S. Small, Secretary.

II. COMMITTEE ON CONTACTANTS

During the current year, the Committee on Contactants has functioned as an advisory committee.

The United States Food and Drug Administration requested the cooperation of the committee in an evaluation of procedures used to establish sensitivity to paraphenylenediamine hair dyes. The following physicians participated in this project: Donald J. Birmingham, George H. Curtis, Max Grolnick, Naomi M. Kanof, Morris Leider, R. L. Mayer, Samuel M. Peck, A. Ros-

tenberg, Jr., W. C. Spain, Karl Steiner, Marion B. Sulzberger, and Raymond R. Suskind. The answers were tabulated and submitted to the Food and Drug Administration on Sept. 16, 1955. The committee received a letter from Dr. A. H. Holland, Jr., in grateful acknowledgment of the report and, as a result of it, the secretary was asked to testify at a hearing in Washington, D. C., on Jan. 6, 1956, during a meeting on the subject which was held under the auspices of the F.D.A.

Officers, Members, and Collaborators.—Dr. Max Grolnick, 555 Ocean Ave., Brooklyn, New York, is the secretary of the committee. The roster of its members and advisers has remained unchanged since the last report.

III. COMMITTEE ON DRUGS

Activities.—The Committee on Drugs has taken a number of steps to enlarge its activities. Letters which outlined the facilities of the committee were sent to the major pharmaceutical manufacturers in the United States. The majority of the companies replied and stated that they would be glad to take advantage of the committee's invitation to submit material for evaluation if and when drugs of interest to us should become available. In addition, the committee has established close liaison with the Division of Medicine of the Food and Drug Administration (through its Associate Medical Director, Dr. Irvin Kerlan) and will participate in this survey of problems which are of interest and concern to allergy.

The committee will increase its efforts and encourage pharmaceutical firms to submit promising compounds to the committee at an earlier stage in their development than in the past. Several inquiries which have been received in recent months appear promising and might be included in the investigative projects scheduled for 1956 and 1957.

The studies carried out during 1955 and 1956 involved the further evaluation of Plimasin, a product of the Ciba Company, Summit, New Jersey, which contains Pyribenzamine, 25 mg., and Ritalin. This is a continuation of the study reported last year, extended to include cases of seasonal allergy. In addition, observations on reducing the amount of Ritalin from 5 mg. to 3 mg. were made.

In July, 1955, Dr. George Babcock, of the Schering Corporation, Bloomfield, New Jersey, asked the committee to evaluate their new steroids, Meticorten (prednisone) and Meticortelone (prednisolone) in various allergic states. The first portion of this study was carried out during the 1955 ragweed pollinating season, and the results were summarized for presentation with the annual report of the committee. Subsequent phases of this study cover the action of these steroids in nonseasonal allergic rhinitis, chronic asthma, urticaria, and eczema.

At its annual session on Feb. 3, 1956, the Research Council voted (1) not to accept drugs for evaluation unless structure and action can be made known

to each member of the subcommittee and (2) to classify drugs in accordance with the specific problems which they present. They are classified as follows:

I. Drugs which are not acceptable because they have no direct application to the field of allergy or because adequate information about their action is already available.

II. Drugs which are acceptable for study. This second group is divided into two categories:

A. Drugs which are generally available, for example:

1. Old drugs with new applications or reduction in side effects, or
2. New drugs which, though already on the market, need a more adequate evaluation of their effectiveness, indications, dosage, or limitations in the treatment of allergic states

B. New drugs not yet available for general use which, after completion of toxicity studies and pharmacologic definition in the laboratory, appear to have promise in the treatment of allergic conditions.

Officers, Members, and Collaborators.—At present, the structure of the committee does not provide for subcommittees, although the apparent interest of the members in an increasing variety of projects might make it necessary to activate subcommittees within the near future. The roster of the committee at present reads as follows: Drs. Sidney Friedlaender (Chairman), Murray Albert, Clarence Bernstein, Theodore B. Bernstein, Earl Benedict Brown, Seymour Crepea, Alex S. Friedlaender, Harold J. Friedman, Frank T. Joyce, Edmund L. Keeney, Saul Malkiel, Dean A. Moffat, Milton M. Mosko, Irving W. Schiller, Sheppard Siegal, Aaron D. Spielman, Walter L. Winkenwerder. In addition to these members, Dr. Alfred Gilman and Dr. Carl Dragstedt have acted as pharmacologic consultants. In April, 1955, because of many other pressing duties, including membership on the Council on Pharmacy and Chemistry of the A. M. A., Dr. Dragstedt asked to be relieved of his position. Dr. Harry Hayes, Professor of Pharmacology, Wayne University College of Medicine, has kindly agreed to become his successor.

IV. COMMITTEE ON FOOD ALLERGY

Activities.—The previous report of the Committee on Food Allergy divided its activities into (1) maintenance of the reaginic bank and (2) studies on the standardization of allergenic extracts. Since then, the committee has singled out two groups of allergens for special consideration: (a) fresh fruits and (b) fish, which are now studied by groups on the West and East Coasts, respectively.

The main concern of the committee at this time is the increasing need for reaginic sera, and the difficulty in obtaining them. The serum supply has been depleted by previous studies and should be replenished as soon as pos-

sible. The committee is considering the setting up of additional collecting stations in Boston, Chicago, Los Angeles, Minneapolis, and New Orleans. Better methods of publicity also will be required to keep our members informed of our needs. Only a limited number of the major allergy clinics have contributed toward our bank. Collection through personal contact, and perhaps through local allergy societies and the Red Cross, might restore our dwindling supplies.

Considerable attention will be given to the improvement of the technical problems of the reaginic bank. Plans include (1) a clear definition of the required types of serum (some of our correspondents seemed to feel that only "hot serum" with high titers would be acceptable to us), (2) a simplification of the forms, and (3) a maintenance of the comparatively low fee for our services.

In recent months, the reaginic bank has been faced with an apparent demand for serum containing reagins to penicillin. Of various individuals and organizations, Dr. Irvin Kerlan of the Food and Drug Administration will probably require the largest amount because his organization is engaged in a study of the effect of penicillin in cow's milk on penicillin-sensitive patients. Members who are called upon to treat penicillin-sensitive patients and who are willing to cooperate in the project are urged to get in touch with Dr. Bernard B. Siegel, Jewish Hospital, Brooklyn, New York.

The addition of fresh fruits and fish to the series of allergens under study is part of the committee's survey of different methods of the preparation of allergic extracts. The antigen or antigens of fish have never been clearly identified. Dr. Eloise W. Kailin and her group will prepare allergenic extracts from selected members of the fish family for direct testing and testing by passive transfer. The available pool of serum of fish-sensitive patients has been assigned to the project, and Dr. Kailin is inviting the cooperation of all members who are acquainted with, or who treat, patients who are allergic to fish.

Officers, Members, and Collaborators.—George I. Blumstein (Secretary), Giacomo R. Ancona (fresh fruits), Katherine L. Bowman (reaginic bank), Mary C. Johnson (allergenic extracts), Eloise W. Kailin (fish), Saul Malkiel (fish), Irwin C. Schumacher (fresh fruits), Bernard B. Siegel (allergenic extracts and reaginic bank), Margaret Strauss-Ballard (allergenic extracts), Richard D. Wiseman (fish).

V. SUMMARY

The interest of the membership in the activities of the Research Council has been gratifying. Suggestions for an expansion of its scope have been made on several occasions. The report shows, for instance, that it might be worth while to initiate a survey of the sensitizing effect not only of penicillin, but of other chemicals which might appear in foods. While this is clearly a problem of growing importance, it presents staggering technical difficulties. Our discussion with Dr. Irvin Kerlan of the Food and Drug Administration presents, however, a declaration of intention and perhaps a first step in our efforts to participate on a limited scale in future investigations of this type.

The analysis of the activities of the Research Council during the past year suggests that it might, perhaps, be well to divide the Research Council into (1) active and (2) advisory committees. Advisory committees would include committees on legislative liaison, nomenclature, and, perhaps, Dr. Grolnick's Committee on Contactants which has functioned more or less as a committee of consultants since 1953. A division of this kind might be an effective step in the development of the Research Council toward the fulfillment of its major stated objectives: (1) to contribute to the exchange of ideas among individuals and groups of related interests and (2) to encourage and direct group research.

Respectfully submitted,

Max Samter, M.D.
Secretary.

REPORT OF THE COMMITTEE ON NEW DRUGS OF THE RESEARCH COUNCIL OF THE AMERICAN ACADEMY OF ALLERGY, 1955-56

STUDIES ON PREDNISONE AND PREDNISOLONE IN RAGWEED POLLINOSIS*

DURING the past year, Dr. George Babcock, Jr., Director of Research, Schering Corporation, asked the Committee to study the clinical effects of their new steroids, Meticorten (prednisone) and Meticortelone (prednisolone), in various allergic syndromes. While some observations had already been reported by individual investigators and others were in the process of being carried out, it was felt that a combined study could obtain an objective picture of the efficacy, dosage range, and side effects of these analogues of cortisone and hydrocortisone at a relatively early period in their use. Collaborators in the first phase of the evaluation were the following Committee members: Drs. Theodore B. Bernstein, Evanston, Illinois; Seymour Crepea, Madison, Wisconsin; Alex S. Friedlaender, Detroit, Michigan; Harold J. Friedman, Cleveland, Ohio; Frank T. Joyce, Denver, Colorado; Irving W. Schiller, Boston, Massachusetts; Aaron D. Spielman, New York, New York; Walter L. Windenwerder, Baltimore, Maryland; and Sidney Friedlaender, Detroit, Michigan, secretary.

Plan of Study.—It was decided first to observe the effects of the steroids in pollinosis, and subsequently to study their influence on other allergic syndromes. The present, or hay fever, phase of the report covers treatment of 221 patients who were selected primarily because of symptoms of ragweed hay fever, although approximately one-half also had associated asthma. The limitations and hazards of steroid therapy being well recognized by all investigators, only those patients with severe symptoms were selected for the study.

One hundred ten patients, including twenty children aged 5 to 12 years, received prednisone initially, while 111 patients, including twenty-one children, were treated with prednisolone. Some in each group were later transferred to the other steroid in order to make comparisons in the same patient. Treatment was carried out for periods ranging from two to fifty-four days (Table I). The average duration of treatment with prednisone was fourteen days, and with prednisolone it was sixteen and one-half days.

The effect on symptoms was graded as excellent (90 to 100 per cent relief), good (75 to 90 per cent relief), fair (50 to 75 per cent relief), and poor (less than 50 per cent relief). One investigator (I) reported cases in tabular form on which results were indicated as: "almost total absence of symptoms" (rated in our results as "excellent"); "presence of only a few mild, but not troublesome, symptoms" (classified here as "good"); and "slight or no relief" (reported here as "poor").

*The prednisone used in this study was supplied as Meticorten, and the prednisolone as Meticortelone by the Schering Corporation, Bloomfield, New Jersey.

TABLE I. DURATION OF THERAPY

DAYS OF TREATMENT	PREDNISONE (NUMBER OF PATIENTS)	PREDNISOLONE (NUMBER OF PATIENTS)
Less than 7	10	6
7 to 14	64	50
15 to 21	16	23
Over 21	20	32

Average duration of treatment:
Prednisone—14 days.
Prednisolone—16½ days.

Since optimum dosage was to be determined, each investigator set the dosage level for his own cases. In most instances, the amounts given were guided by the severity of the individual patient's symptoms, although some investigators set a standard dosage schedule which they adhered to in all of their cases, regardless of severity. The steroids were supplied in 5 mg. tablets and the daily amount was generally administered in four divided doses. For each patient included in the study, an effort was made to obtain the following dosage data: initial daily dose; total dose required for maximum suppression of symptoms; hours of treatment required to achieve optimum effect; daily maintenance dose; or dose below which symptoms recurred or became worse.

TABLE II. RESULTS OF TREATMENT IN HAY FEVER

RESULTS	PREDNISONE*	PREDNISOLONE†
Excellent (90 to 100 per cent relief)	55 (50.0 per cent)	48 (43.2 per cent)
Good (75 to 90 per cent relief)	35 (31.85 per cent)	39 (35.1 per cent)
Fair (50 to 75 per cent relief)	5 (4.5 per cent)	12 (10.8 per cent)
Poor (Less than 50 per cent relief)	15 (13.65 per cent)	12 (10.8 per cent)

*110 patients treated.
†111 patients treated.

Results (Table II).—

Effect on hay fever symptoms: In 110 patients treated with prednisone, results were classed as excellent in fifty-five, or 50 per cent; good in thirty-five, or 31.85 per cent; fair in five, or 4.5 per cent; and poor in fifteen, or 13.65 per cent (Table III).

In 111 patients initially receiving prednisolone, results were excellent in forty-eight, or 43.2 per cent; good in thirty-nine, or 35.1 per cent; fair in twelve, or 10.8 per cent; and poor in twelve, or 10.8 per cent (Table IV).

Effect on associated asthmatic symptoms (Table V): In thirty-seven patients with associated asthma who received prednisone, the effect on asthmatic symptoms was better than on hay fever in seven patients; less helpful in asthma than hay fever in one; and of equal benefit in the remaining twenty-nine. In forty-four patients receiving prednisolone, nine patients experienced more relief of asthma than hay fever; one had more benefit on hay fever than asthma; and thirty-four patients attained equivalent relief of both symptoms.

Comparison of prednisone and prednisolone in the same patient: In sixteen patients in whom both steroids were used interchangeably, equivalent

TABLE III. PREDNISONE—RESULTS OF TREATMENT IN HAY FEVER

INVESTIGATOR	RANGE OF INITIAL DAILY DOSE (MG.)	RANGE OF SUPPRESSIVE DOSES (MG.)	RANGE OF MAINTENANCE DOSES (MG.)	TOTAL PATIENTS TREATED	RESULTS				NUMBER OF PATIENTS EXPERIENCING SIDE EFFECTS
					EXCELLENT	GOOD	FAIR	POOR	
A	10-20	10-80	5-20	11	6	3	0	2	0
B	5-60	20-90	5-20	16	5	10	1	0	2
C	15-30	10-100	5-10	9	6	2	1	0	1
D	30-45	--	10-35	8	3	0	2	3	0
E	20-30	15-30	10-30	6	3	1	0	2	1
F	15-60	10-140	5-25	8	4	3	0	1	2
G	15-60	10-100	5-25	7	3	2	1	1	1
H	20-40	20-115	5-15	11	9	2	0	0	0
I	10-20	10-60	--	34	16	12	--	6	5
Total				110	55 (50.0%)	35 (31.85%)	5 (4.5%)	15 (13.65%)	12 (10.9%)

TABLE IV. PREDNISOLONE—RESULTS OF TREATMENT IN HAY FEVER

INVESTIGATOR	RANGE OF INITIAL DAILY DOSE (MG.)	RANGE OF SUPPRESSIVE DOSES (MG.)	RANGE OF MAINTENANCE DOSES (MG.)	TOTAL PATIENTS TREATED	RESULTS				NUMBER OF PATIENTS EXPERIENCING SIDE EFFECTS
					EXCELLENT	GOOD	FAIR	POOR	
A	15-20	20-140	5-20	17	3	6	3	5	0
B	15-40	15-80	5-20	15	4	8	2	1	0
C	10-30	5-60	5-20	9	6	2	1	0	0
D	30-35	--	--	8	1	4	1	2	1
E	20-30	15-30	5-30	9	8	1	0	0	2
F	20-60	20-100	10-30	14	6	4	3	1	1
G	20-60	20-100	10-30	14	7	3	2	2	1
H	15-20	20-95	5-15	11	8	3	0	0	0
I	10-20	--	--	14	5	8	--	1	1
Total				111	48 (43.2%)	39 (35.1%)	12 (10.8%)	12 (10.8%)	6 (5.4%)

TABLE V. EFFECT ON ASTHMA AND HAY FEVER SYMPTOMS IN SAME PATIENT

EFFECT	PREDNISONE	PREDNISOLONE
Same effect on asthma and hay fever	29	34
Better effect on asthma than hay fever	7	9
Better effect on hay fever than asthma	1	1
Total patients	37	44

results at the same dosage levels were reported in fourteen cases. One patient appeared to achieve a greater response from prednisone, while another was more favorably benefited by prednisolone.

Dosage (Table VI).—

Initial daily dose: The initial daily dose of both prednisone and prednisolone varied from 10 to 60 mg. Most adult patients received 20 to 40 mg. as the initial daily dose, with an average of 27.5 mg. for prednisone and 29.5 mg. for prednisolone. In children aged 5 to 12 years, the dosage range for both steroids was 10 to 40 mg., with the average initial daily dose about 75 per cent of that given to older patients.

TABLE VI. DOSAGE DATA

	PREDNISONE	PREDNISOLONE
Range of initial daily doses	10 to 60 mg.	10 to 60 mg.
Average initial daily dose	27.5 mg.	29.5 mg.
Range of suppressive doses	10 to 140 mg.	5 to 140 mg.
Average suppressive dose	45.8 mg.	41.1 mg.
Range of maintenance doses	2.5 to 40 mg.	2.5 to 30 mg.
Average maintenance dose	10.5 mg.	12.7 mg.
Range of time for optimum relief	6 to 120 hours	4 to 168 hours
Average time for optimum relief	40.2 hours	33.5 hours

Dose and time required for maximum suppression of symptoms: For patients achieving good to excellent results from prednisone, the dose required to suppress symptoms varied from 10 to 140 mg., with an average of 45.8 mg. Optimum relief was reported as occurring in from 6 to 120 hours, the average time being 40.2 hours.

In the case of prednisolone, the suppressive doses ranged from 5 to 140 mg., with an average of 41.1 mg. Optimum relief occurred in from 4 to 168 hours, with an average of 33.5 hours.

While no definite relationship between the size of the initial dose and time for optimum relief was determined, there was some indication that the onset of relief of symptoms tended to occur somewhat earlier when the initial doses were higher.

Maintenance dose: Following the attainment of a maximum suppressive response, most investigators, in conformity with past experience in the use of steroids, gradually tapered the dose to the lowest point able to maintain the patient's improvement and below which symptoms recurred or became worse. Where this dose was possible to determine, it ranged from 2.5 to 40 mg. for prednisone and from 2.5 to 30 mg. for prednisolone. The greatest number of cases fell within the range of 5 to 20 mg., with an average of 10.5 mg. for prednisone and 12.7 mg. for prednisolone.

Weight, Blood Pressure, and Urine.—Urinalysis for sugar was carried out before and at conclusion of treatment, but no cases of glycosuria were reported. Weight and blood pressure recordings were made before and after treatment wherever possible.

TABLE VII. WEIGHT GAIN WITH PREDNISONE

WEIGHT GAIN (LBS.)	INITIAL DOSE (MG.)	SUPPRESSIVE DOSE (MG.)	MAINTENANCE DOSE (MG.)	DURATION OF TREATMENT (DAYS)
10	35	--	10-20	20
7	30	60	10	4
5	40	30	20	19
5	30	--	--	14
4	60	140	25	9
4	30	30	15	12
4	20	60	10	28
4	20	60	10	9
4	20	30	5	8
4	30	100	5	27

Of seventy-three patients receiving prednisone, sixty-three, or 86.3 per cent, showed weight variations (up or down) of 3 pounds or less. Ten patients, or 7.3 per cent, gained from 4 to 10 pounds (Table VII). Of ninety-one patients on prednisolone, eighty-five, or 93.5 per cent, gained or lost 3 pounds or less. Six patients, or 6.6 per cent, recorded weight gains of 4 to 6 pounds. There was no direct relationship evident between weight gain, steroid dosage, or duration of therapy in these patients (Table VIII).

TABLE VIII. WEIGHT GAIN WITH PREDNISOLONE

WEIGHT GAIN (LBS.)	INITIAL DOSE (MG.)	SUPPRESSIVE DOSE (MG.)	MAINTENANCE DOSE (MG.)	DURATION OF TREATMENT (DAYS)
6	20	10	5.0	14
5	20	20	7.5	26
4	40	40	10.0	22
4	50	30	20.0	30
4	30	--	20.0	13
4	50	40	20.0	30

Of sixty-seven patients receiving prednisone, where blood pressure was recorded before and after therapy, seventeen showed increases in systolic or diastolic pressure of 5 mm. or less, while fifteen showed similar decreases. The readings in thirty-five others were unchanged.

Of eighty-four patients on prednisolone therapy, twenty-three showed increases of 5 mm. or less, while twenty showed equivalent decreases, and forty-one others were unchanged.

Side Effects.—Untoward effects associated with the use of prednisone in this series were recognized in twelve patients, or 10.9 per cent. Increased central nervous system stimulation was most frequent; next most common was upper gastrointestinal distress; while muscular pain, headache, palpitation, frequency, and urgency of urination were also recorded. Side effects were severe enough to require discontinuation of treatment in four of these cases (Table IX).

TABLE IX. SIDE EFFECTS (PREDNISONE)

PATIENT	AGE (YEARS)	INITIAL DOSE (MG.)	SUPPRESSIVE DOSE (MG.)	MAINTENANCE DOSE (MG.)	DURATION OF TREATMENT (DAYS)	SYMPTOM	REQUIRED DISCONTINUATION OF TREATMENT
T. K.	7	10	--	--	12	Stimulation	No
G. C.	12	15	--	--	1	Stimulation	Yes
S. K.	50	20	--	--	5	Stimulation, headache	Yes
L. L.	16	20	--	--	12	Stimulation	No
A. B.	20	30	--	--	3	Pyloric pain	Yes
D. I.	15	50	30	15	22	Tremor of hand; pains in muscles of legs	No
G. B.	46	50	70	10	30	Jitteriness and nervousness at high doses	No
A. J.	25	30	60	10	4	Epigastric pain	Yes
C. T.	19	60	140	25	9	Palpitation	No
J. M.	24	40	60	10	10	Esophagitis	No
M. M.	6	15	60	5	18	Frequency and urgency of urination	No
H. M.	6	10	--	--	12	Stimulation	No

TABLE X. SIDE EFFECTS (PREDNISOLONE)

PATIENT	AGE (YEARS)	INITIAL DOSE (MG.)	SUPPRESSIVE DOSE (MG.)	MAINTENANCE DOSE (MG.)	DURATION OF TREATMENT (DAYS)	SYMPTOM	REQUIRED DISCONTINUATION OF TREATMENT
J. K.	12	35	--	--	14	Increased urinary output	No
I. M.	44	20	20	5-20	12	Euphoria	No
C. C.	35	30	30	30	21	Anterior chest pain at higher doses	No
A. A.	38	50	50	--	8	Tachycardia, urinary frequency	Yes
T. M.	32	20	60	15	20	Gastrointestinal disturbance	Yes
E. M.	26	20	--	--	2	Nausea	Yes

Six patients, or 5.4 per cent, experienced side effects related to prednisolone therapy. These complaints consisted of gastrointestinal disturbances, anterior chest pain, euphoria, tachycardia, urinary frequency, and polyuria. Cessation of treatment because of side effects was necessary in three patients (Table X).

Comment.—The participants in this study found that prednisone and prednisolone were very effective agents in temporarily alleviating the symptoms of ragweed hay fever and asthma. In common with older steroids, symptoms recurred rather promptly on withdrawal or reduction below a maintenance level. While the effective dosage of these newer analogues on a milligram basis was one-fourth to one-fifth that of hydrocortisone and cortisone, most untoward side effects generally associated with steroid therapy were encountered at such therapeutic levels. The principal advantage of prednisone and prednisolone appears to lie in a decreased tendency to retain sodium and water. Although side effects may be expected to be less severe in the case of patients on short-term steroid therapy at lower dosage levels, as represented in this series, the occurrence of major side effects during short-term prednisone and prednisolone therapy, such as reactivation of peptic ulcer, gastrointestinal bleeding, disturbances in carbohydrate metabolism, and cardiac arrythmias, are already within the experience of members of this Committee. It is likely that other metabolic and long-term complications will be comparable to those associated with cortisone and hydrocortisone. It is felt, therefore, that treatment of hay fever with these newer, as well as older, steroids should be limited only to those severe cases that are unresponsive to desensitization or fail to achieve relief from the more conservative forms of symptomatic therapy.

PLIMASIN (CIBA)

The current report represents an extension of last year's study* of Ciba's Plimasin, a combination of Pyribenzamine, 25 mg., and the analeptic drug Ritalin [phenyl-piperidyl-(2)-acetic acid methylester], 5 mg. At that time. treatment with Plimasin covered cases of nonseasonal allergic rhinitis, urticaria, and the dermatitides. The conclusions drawn from that report were that Plimasin was an effective antihistaminic combination producing a minimum of side effects, the most frequent of which was central nervous system stimulation. The usual sedation seen with antihistaminic drugs was markedly reduced.

The present study was continued principally to include the treatment of patients with seasonal allergic rhinitis. The following members of the Committee on New Drugs contributed cases to this portion of the report: Drs. Murray Albert, Brooklyn, New York; Earl B. Brown, New York, New York; Seymour Crepea, Madison, Wisconsin; Alex S. Friedlaender, Detroit, Michigan; Harold J. Friedman, Cleveland, Ohio; Frank T. Joyce, Denver, Colorado; Saul Malkiel, Worcester, Massachusetts; Milton M. Mosko, Chicago, Illinois; Aaron D. Spielman, New York, New York; Walter Winkenwerder, Baltimore, Maryland; and Sidney Friedlaender, Detroit, Michigan, secretary.

*Report of the Committee on New Drugs of the Research Council, American Academy of Allergy, J. ALLERGY 26: 379, July, 1955.

A total of 354 case reports were submitted representing 294 patients treated with Plimasin and 60 patients treated with a modified preparation containing 3 mg. instead of 5 mg. of Ritalin, together with 25 mg. of Pyribenzamine. The purpose of the latter modification was to determine whether a reduction in the dose of analeptic drug would decrease the incidence of central stimulation, without an increase in sedation, while still producing an equivalent clinical effect.

TABLE XI. CLINICAL EFFECTIVENESS IN SEASONAL ALLERGIC RHINITIS

DRUG USED	NUMBER OF PATIENTS	PATIENTS SHOWING 50 PER CENT OR GREATER IMPROVEMENT
Plimasin	255	185 (72 per cent)
Pyribenzamine*	104	73 (70 per cent)
Ritalin	42	11 (26 per cent)
Pyribenzamine 25 mg. and Ritalin 3 mg.	48	42 (87 per cent)

*Pyribenzamine or other antihistaminics.

TABLE XII. COMPARATIVE EFFECTIVENESS WITH OTHER ANTIHISTAMINICS IN SAME PATIENTS WITH SEASONAL HAY FEVER

RESULTS	NUMBER OF PATIENTS
Plimasin	
(1) Better than others	9
(2) Worse than others	2
(3) Same as others	78
Pyribenzamine, 25 Mg., and Ritalin, 3 Mg.	
(1) Better than others	3
(2) Worse than others	0
(3) Same as others	8

TABLE XIII. SUMMARY OF SIDE EFFECTS

	DRUG USED			
	PLIMASIN	PYRIBENZAMINE*	RITALIN 5 MG.	PBZ, 25 MG., AND RITALIN, 3 MG.
Total number of patients	294	143	61	60
Type of Side Effects				
Sedation	16	59	2	4
Central stimulation	37	4	14	6
Gastrointestinal	7	2	1	1
Number of patients with side effects	60 (20.4%)	65 (45.4%)	17 (27.9%)	11 (18.3%)
Number of patients without side effects	234	78	44	49
Total number of moderate to severe side effects	25 (8.5%)	30 (20.9%)	7 (11.7%)	4 (6.6%)

*Pyribenzamine or other antihistaminics.

Plimasin was administered to 255 patients with seasonal allergic rhinitis and to thirty-nine patients with other allergic conditions. The dosage varied from two to eight tablets daily, depending on the severity of symptoms, individual tolerance, and clinical effectiveness.

The modified Plimasin preparation, containing 3 mg. of Ritalin, was administered to sixty patients, of whom forty-eight had hay fever and the remainder other allergic syndromes.

Pyribenzamine or another antihistaminic was used at some time for comparison in 104 of these patients. Ritalin tablets, 5 mg., were substituted at one time or another during the study in sixty-one patients, of whom forty-two had seasonal allergic rhinitis.

Results.—The clinical effectiveness of these preparations in seasonal allergic rhinitis is summarized in Tables XI and XII. The results indicate that Plimasin, as well as the modified form containing only 3 mg. of Ritalin, are highly effective in affording symptomatic relief of hay fever symptoms. Their effect compares very favorably with therapeutic doses of other available antihistaminic agents. While a greater percentage of the smaller group treated with modified Plimasin benefited, more extensive use would be necessary to evaluate this properly. Ritalin alone, in doses of 5 mg., appeared to exert limited effectiveness in cases of seasonal hay fever.

The results in patients treated for other allergic conditions, such as nonseasonal rhinitis, urticaria, and eczema, closely approximated those reported in the Committee's prior study. Twenty-four out of thirty-nine patients, or 61.5 per cent, achieved appreciable symptomatic relief from Plimasin, while eight out of twelve patients, or 66 per cent, were similarly benefited from the modified form of Plimasin.

Side Effects.—The side effects occurring from the preparations used in the study are summarized in Table XIII. It is seen that the untoward effects from the Ritalin-containing combinations were substantially less in number and altered in type from those usually seen with antihistaminic drugs. In common with the prior Committee report, central stimulation was the most frequent side effect in the case of Plimasin. This was somewhat less marked when Ritalin in the preparation was reduced to 3 mg. More experience with the modified preparation is required to assess this thoroughly.

Summary and Conclusions.—Plimasin is an effective antihistaminic combination for the treatment of seasonal allergic rhinitis. Its principal advantage appears to lie in a lowered incidence of side effects, principally sedation. There is an increase in central nervous system-stimulating effects, which may be an advantage in daytime medication. Limited study with a modification of Plimasin containing 3 mg., instead of 5 mg., of Ritalin together with 25 mg. of Pyribenzamine indicated that the dose of analeptic drug may be somewhat reduced without reducing the clinical effectiveness. More extensive use of this modification of Plimasin is necessary to determine whether the incidence of side effects is appreciably altered by this change.

Respectfully submitted,

Sidney Friedlaender, M.D.
Secretary, Committee on New Drugs
of the Research Council, American
Academy of Allergy.

Obituaries

AT THE twelfth annual meeting of the American Academy of Allergy, the Obituary Committee reported with regret the deaths of the following members and fellows of the Academy.

DOROTHY M. LANG, M.D., White Plains, New York, born 1898. Dr. Lang was elected to membership in the American Academy of Allergy in 1947. She was an attending physician in pediatrics at the White Plains Hospital and at the Grasslands Hospital, and a consultant in pediatrics at the Terrytown Hospital. Dr. Lang was a member of the American Academy of Pediatrics.

JOHN PETERS, M.D., Oak Park, Illinois, born 1889. Dr. Peters had been a fellow of the American Academy of Allergy since 1947. He was also a fellow of the American College of Physicians and the American College of Allergists, and served in both World Wars.

RALPH BOWEN, M.D., born in 1898, died Jan. 31, 1956, of coronary occlusion. He had been a fellow of the American Academy of Allergy since 1950. Dr. Bowen obtained his M.D. degree from Iowa State University in 1924. He was associate professor of medicine at Baylor University College of Medicine, lecturer in pediatrics at the University of Texas, Medical Branch, and chief of allergy at Texas Childrens Hospital. Dr. Bowen was a past-president of the Southwest Allergy Forum and one of the founders of the American College of Allergists. He was a past-president of the Southern Medical Association, Allergy Division.

JOSEPH SKWIRSKY, M.D., Newark, New Jersey, died in September, 1955. Dr. Skwirsky became a member of the Society for the Study of Asthma and Allied Conditions in 1940 and was elected to fellowship in the American College of Allergy in 1948. He was a former president of the New Jersey Diabetic Association and had a special interest in diabetes and metabolic diseases.

Announcements

Third European Congress of Allergology

The Third European Congress of Allergology, organized by the Italian Society of Allergology, will be held in Florence, Italy, Sept. 12 through 15, 1956. The times and meeting places will be announced later.

The program will be divided into principal sessions and subordinate sessions.

The main topics for discussion at the principal sessions are:

1. The Genesis and Localization of Antibodies.
2. Allergy and the Neurovegetative System.
3. Factors Involved in the Increase of Allergic States at the Present Time.

The subordinate sessions will be devoted to discussions on:

1. Allergological Classification and Terminology.
2. Standardization of Allergenic Extracts.
3. The Mechanism of Retarded (Delayed) Reactions.

The official languages will be Italian, French, English, and German. A translation service will be organized.

Registration for the Congress will enable participants to present papers bearing on the chosen subjects and to enter the discussions, to receive the *Proceedings* of the Congress, and to take part in the exhibitions.

The organizing committee will publish the final program, and will announce the registration fee and other pertinent notices at a later date.

The officers of the Congress are:

Hon. President, Prof. Cesare Frugoni.
President, Prof. Antonio Lunedei.
Secretary General, Prof. Umberto Serafini.

For further information and for registration, address the president or the secretary general, in care of:

Istituto di Patologia Medica
Viale Morgagni
Florence, Italy.

Fellowship and Residency in Allergy

An opening is available for a fellowship in allergy at Northwestern University Medical School. This provides a combination of clinical and investigative work in allergy for training in specialization and is approved for credit by the Board of Internal Medicine and the subspecialty Board of Allergy. Annual stipend is $3,000.

A residency in allergy is also available at the Veterans Research Hospital. This residency provides for service equally divided at the Hospital and the Medical School.

For details and application blank, write to Dr. S. M. Feinberg, Northwestern University Medical School, 303 E. Chicago Ave., Chicago 11, Illinois.

Approval of Residencies in Pediatric Allergy

The American Board of Pediatrics, through its subspecialty Board of Allergy, and the Council on Medical Education and Hospitals of the American Medical Association are developing a program for the approval of residency programs in allergy in preparation for certification in that subspecialty.

Applications for approval should be submitted to the Executive Secretary of the American Board of Pediatrics, Dr. John McK. Mitchell, 6 Cushman Rd., Rosemont, Pennsylvania.

Correspondence

To the Editor:

Those of us who have watched the field of allergy grow from its inception are continuously impressed by its rapid progress. From a specialty limited at first to two diseases—hay fever and asthma—its scope has become so widened that today it embraces a multitude of varied conditions involving all types of tissues and organs. Conditions whose etiology formerly was obscure or unproved now are known to have a basic allergic etiology or pathogenesis; for example, periarteritis nodosa and idiopathic purpura. In some of these conditions, proof of the allergic etiology is definite, whereas in others it is only presumptive. Regardless of these considerations, however, allergists generally agree that, because of the factor of hereditary predisposition and proved allergic etiology, four conditions deserve the designation of "atopic": namely, hay fever, asthma, migraine, and atopic dermatitis. But recently serious doubt has been cast upon the propriety of designating the condition now termed atopic dermatitis as "atopic." This seemed to me to be of sufficient importance to warrant more detailed discussion by allergists, and is the reason for the present communication.

Although older physicians, especially dermatologists, long have known that patients subject to "neurodermatitis" or "flexural eczema" had familial tendencies or diatheses, it remained for Sulzberger and Coca, in 1934, to take this condition out of the dermatologic scrapheap of "eczemas" by proving its allergic basis and by properly designating the condition as "atopic dermatitis." Allergists and dermatologists alike hailed this as a notable advance and were willing to accept both the designation and the proof of its etiology, but when many patients failed to respond to what was regarded as proper allergy treatment and the expected "cures" failed to materialize, considerable doubt began to arise as to whether allergy had anything to do with this disease. This apparently now has reached such proportions, at least in the minds of many dermatologists and even some allergists, as to threaten to nullify the original advances initiated by the report of Sulzberger and Coca, and to turn back the clock of progress by several decades. Evidence of this doubt is quite apparent in a recent small monograph entitled *Atopic Dermatitis,* edited by Rudolph Baer and published by the J. B. Lippincott Company. It contains a series of articles previously published in the *American Practitioner* and written entirely by dermatologists. After reviewing it, I was at a loss to account for its purpose and was concerned about its possible influence upon those who are called upon to treat patients with atopic dermatitis. After reading the conflicting opinions contained in this monograph, the reader is left in a complete state of confusion. He is led to believe that the condition termed "atopic dermatitis" may not have an atopic basis and is actually now only a term of convenience; also that even though allergy may at times be a factor, the actual etiology is unknown and indefinite, that the real pathogenesis is one of pruritis (the causes being varied and not always determinable), the lesion itself being secondary and due to scratching, that allergic studies, including skin tests and diet trial, are in most instances useless and uninformative, and that allergy treatment except in the occasional case is of no value whatsoever. How these authors can write these things in the face of the abundant clinical evidence to the contrary is a mystery. How they can ignore the remarkable responses of eczematous infants to the withdrawal of eggs, milk, or other foods to which they are allergic or the responses of older children and adults allergic to inhalant allergens to properly instituted allergic treatment is difficult to under-

stand. It likewise is hard to comprehend how they can ignore the mass of evidence proving the etiological relationship to atopic dermatitis of important inhalants like silk (Sulzberger and Vaughan), wool (Osborne, Tuft), house dust (Tuft et al.), pollens, especially ragweed (Rowe, Feinberg, Tuft et al.) and atmospheric molds (Tuft et al., Jillson). To state that these allergens are uncommon factors is to ignore proved evidence or to indicate inadequate clinical experience.

It seems to me that those who would want to eliminate the designation of "atopic" apparently have had little or no experience with other allergic diseases or the general subject of allergy, for there is not a single objection voiced in these articles against the proof of allergic etiology that does not apply equally to bronchial asthma. There are many asthmatics who have positive skin tests which are "false" or without clinical significance; there are also many who do not respond to diet exclusion or diet trial or to desensitization, or in whom no specific proof of a definite etiology is available (e.g., the so-called "intrinsic" or "infectious" type). And yet, while allergists might argue about the etiology, none would claim that because of these deficiencies asthma should not be regarded as an atopic disease. In fact, as I have pointed out in previous publications on this subject, atopic dermatitis and bronchial asthma have so many characteristics in common that the former may be designated as "asthma of the skin." This is not appreciated by the dermatologist because he does not (and should not be expected to) know about asthma or allergic diseases in general. By the same token, since atopic dermatitis is only a local manifestation of a general sensitization, the dermatologist should not be expected to be able to treat the allergic phase of this disease any better than if he were asked to treat bronchial asthma, even though he undoubtedly is more expert than the allergist in managing the local and topical treatment of the patient with atopic dermatitis. There would seem to be no more justification for a dermatologist to treat a patient with atopic dermatitis without the aid of an allergist than there would be for an allergist to treat a case of pemphigus, about which he knows nothing, or of the infected pustules of an eruption due to drug allergy. No dermatologist would undertake the management of a patient who comes to him with what he diagnoses as purpura. He would immediately call in a hematologist either to assist him or to take charge of the case completely. Why, then, should not the same procedure be followed in patients with atopic dermatitis where proper allergy (internal) care often is more essential to a favorable outcome than much of the local therapy given by the dermatologist? If this were done more regularly, then there would be fewer and fewer reports of failures in treatment. It is time, I believe, for allergists to assert their prerogative in relation to atopic dermatitis and to inform practitioners and public alike that such patients will fare better if they are placed under the care and guidance of an allergist, with or without the assistance of a dermatologist.

Louis Tuft, M.D.
1530 Locust St.
Philadelphia 2, Pa.

Book Reviews

Pathologic Physiology. By W. A. Sodeman. Second edition, Philadelphia, W. B. Saunders Company, 1956.

Most advances in modern therapeutics have been based on knowledge concerning the basic mechanisms responsible for disease processes. There is good reason, therefore, for more and more stress to be placed on understanding the causes of disease, and less on empirical therapeutics.

This volume reflects this trend in medicine by emphasizing etiology of disease rather than treatment. Accordingly, it should be considered as a supplement to the standard textbooks of medicine.

It is composed of separate sections on genetics, metabolism; infection and allergy; physical, chemical, and toxic agents; the circulatory, respiratory, and digestive systems; the urinary tract; hematology; locomotor system; and nervous system. As would be expected when twenty-five authorities have contributed to this edition, there is much variation in style and in the type of material covered. In this edition, most sections contain many changes from the previous edition and have been rearranged considerably. The editor has added some new introductory remarks on pathologic physiology, and the sections on genetics and neurology are completely new.

The section on allergy is not written for the specialist in the field, and might be of most value as a reference for physicians without special training in allergy. It provides a concise and well-organized review of many immunologic principles, although bibliographic references are quite incomplete.

All physicians should find the sections on pulmonary physiology and lung diseases of special value; they are unusually well presented, and provide material which is not adequately covered in most allergy texts. Immuno-hematology is discussed at considerable length in the section on the blood and deserves careful reading.

There are several deficiencies in this volume, none of them serious. The general adaptation syndrome, with its many unproved hypotheses, is discussed in much detail and takes up a disproportionate amount of the section on endocrinology. Yet there is no discussion of mechanisms responsible for the most important pharmacologic effects of adrenocortical steroids, namely, suppression of inflammatory reactions and fibroblastic proliferation. The relationship of the Type 12 streptococcus to the appearance of acute glomerulonephritis appears nowhere in this book and more information concerning the so-called "collagen diseases" would have been desirable.

This volume should not take the place of textbooks, either on physiology or on medicine. Also, none of its sections can possibly be complete enough to take the place of specialty monographs. However, it should be especially valuable for the physician who desires some fundamental knowledge of subjects outside his own specialty, and it is in this regard that the book is most recommended.

Histamine, Its Role in Anaphylaxis and Allergy. By Rocha e Silva, M. Springfield, Illinois, Charles C Thomas, 1955, 264 pages. Price, $7.50.

In the past half-century, a stupendous number of investigations concerning the role of histamine in animal metabolism and disease have appeared. These studies have involved,

or have been outgrowths of, work in diverse biologic fields, some only remotely related to each other. Furthermore, many studies concerning histamine have been contradictory or have not been confirmed by further investigations.

This monograph by an internationally known authority on histamine is remarkable in covering this difficult field. Not only are the known facts and conjectures concerning histamine formation, tissue binding, liberation, and metabolism discussed at length, but detailed sections are devoted to related topics, such as histaminase, antihistaminic drugs, and other possible causes of anaphylactic shock, such as peptone, trypsin, and animal venoms.

In some ways, this monograph is almost too complete; except in the discussion of leukotoxin, there is little critical discussion of the work which is presented. In spite of the apparently complete coverage of this book, the findings of Valentine and Lawrence on the relationship of histamine to leukocytes in normal and leukemic blood are not mentioned. Furthermore, there is little organized discussion of histamine assay methods, even though assay methods for histaminase were discussed in some detail and adequately indexed. No mention was made of the sensitive colorimetric method for histamine determinations which was first reported by McIntire in 1950.

The author's observations on the clinical efficacy of trisodium citrate in allergic dermatoses have not as yet been confirmed by others. The section relating histamine to clinical allergy is, of necessity, brief and conjectural.

In comparison to the amazing scope and exhaustive discussion on almost every topic in this monograph, the above-mentioned deficiencies are only minor. This treatise, with its description of many classic and provocative investigations, and with an extensive bibliography, should be of great value to experimental workers interested in histamine, and at times should provide valuable reference material for the clinical allergist.